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Supporting Associates for 1959 ---------- 425

International Association for Dental Research. Announcement of 1960 General Meeting ---------- 426

Editorial Views and News ---------- 427

Autoradiographs of Solder Joints With Fluorine[18]. Saul M. Bien and Herbert D. Ayers, Jr., New York, N. Y. ---------- 428

The Distribution of Nitrogen in Human Enamel. Ardis Savory and Finn Brudevold, Rochester, N. Y. ---- 436

Distribution of Some Enzymes in Soft Tissues of the Oral Cavity. O. Svoboda, Z. Lojda, and M. Skach, Prague, Czechoslovakia ---------- 443

Dental Conditions of Preschool Children (One to Six Years of Age) in Szeged, Hungary. K. Toth and I. Szabó, Szeged, Hungary ---------- 451

Comparative Effects of Fluorides on Mice Femurs. Harold S. Fleming, Washington, D. C. ---------- 464

The Relative Local Anesthetic Potencies of Procaine, Oracaine, Kincaine, and Xylocaine. Stanley C. Harris and Robert C. Worley, Chicago, Ill. ----- 477

(Contents continued on inside back cover)

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): The association has been established to promote broadly the advancement of research in all branches of dental science and in the related phases of the sciences that contribute directly to the development of oral health service, and as a whole; and further, to encourage and facilitate cooperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology to the end that dentistry may render cumulatively more perfect service to humanity.

Membership: Total (October, 1958), 938. *Eligibility (Const., Art. III, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to oral health, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Nomination blanks for membership in this Association may be obtained on request from the Secretary-Treasurer.

Endowment fund of the International Association for Dental Research (as of October, 1958): $1,891.01. There have been no expenditures from this fund.

THE JOURNAL OF DENTAL RESEARCH

This JOURNAL is an official publication of the International Association for Dental Research. It is dedicated to the dissemination of knowledge pertaining to the oral cavity, its contents and contiguous parts, and its relation to and effect on the total human organism. The JOURNAL publishes original investigations in these and related fields. Articles are accepted only if they are contributed solely to this JOURNAL.

The JOURNAL is published bimonthly. Subscription price for the volume (6 copies beginning with the January-February issue) is as follows: United States and its possessions, $10.00; (Students, $5.00). Canada, Latin America and Spain, $10.50; (Students, $5.50). Other countries $11.00; (Students, $6.00). Single copies $2.50, postpaid. The subscription fee for members of the IADR is included in the yearly dues paid to the Association. The price of volumes and single numbers of earlier years will be quoted upon request to Dr. David F. Mitchell, University of Indiana, 1121 W. Michigan St., Indianapolis, Indiana.

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All manuscripts and correspondence should be addressed to the Editor: Dr. F. J. Orland, Zoller Dental Clinic, University of Chicago, 950 E. 59th Street, Chicago 37, Illinois.

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*These were the Supporting Associates who had made contributions in 1959 at press time to the I.A.D.R. Treasury for support of the JOURNAL. These contributions are in multiples of one hundred dollars.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
ANNOUNCEMENT OF 1960 GENERAL MEETING

The 38th General Meeting of the International Association for Dental Research will convene at the Morrison Hotel, Chicago, Illinois, on Thursday, Friday, Saturday, and Sunday, March 17, 18, 19, and 20, 1960. The Dental Materials Group of the Association will meet on Friday, Saturday, and Sunday, March 18, 19, and 20, 1960. The American Association of Dental Schools will meet at the same place beginning Monday, March 21, 1960.

Notice to Prospective Program Participants.—Abstracts of items for the program must be limited to *250 words,* including title, authors' names, etc. *Three copies* are required. The abstract as received must be suitable for insertion, without editing, in the Preprinted Abstracts which will be sent to members before the meeting. This will not constitute a publication, but is only a convenience for the meeting. The abstracts will be published in the JOURNAL OF DENTAL RESEARCH after the meeting as in the past.

There will be no Read-by-Title section of the program. Instead, the editor is accepting short research notes for early publication in the JOURNAL OF DENTAL RESEARCH.

Abstracts entered in the *Edward H. Hatton Award Competition* should conform to the specifications stated on the opposite page under "Editorial Views and News" and must be clearly marked as entries for the competition.

The deadline for receipt of program items will be *November 30, 1959.* An abstract received after that date will probably not be included in the preprinted abstracts and will appear on the program only if a place can conveniently be found for it.

DAN Y. BURRILL, *Secretary-Treasurer*
Northwestern University Dental School
311 E. Chicago Avenue
Chicago 11, Illinois

EDITORIAL VIEWS AND NEWS

As mentioned in the last issue of the JOURNAL, the Hatton Award Competition has attracted a significant amount of interest. In fact, some of the papers presented in the Competition were among the best at the San Francisco Meeting. It is therefore fitting to let potential participants in the 1960 Chicago meeting know of the rules.

Revised rules governing the Edward H. Hatton Award Competition for the I.A.D.R.—

1. The Edward H. Hatton Award Competition is for novice research workers. A novice for this purpose is a person who has not presented a research paper before any national organization or published a research report in a national scientific journal.
2. The paper must be presented by the novice at an annual meeting of the I.A.D.R.
3. The paper to be considered for the Hatton Award must be the work of one or more novices. (Note: Persons who are not novices and who have acted in advisory capacities *only* should not be named as authors since the naming of a person not a novice as an author will disqualify the paper.)
4. *Six copies* of the paper and *six copies* of a biographical sketch of each author named on the paper must be submitted to the chairman of the Hatton Award Committee through the Secretary of the I.A.D.R. not less than 30 days before the annual meeting of the I.A.D.R., for review. (Note: This requires *six copies of the full paper,* not just the abstract.)
5. The decisions of the Committee in the selection of winners shall be final.
6. Authors who are eligible to compete for the award should state that fact in a note attached to the abstract submitted to the Secretary for the program committee.
7. The following items will be given consideration by the committee in judging each paper presented: (1) originality, (2) scientific value, (3) accuracy of data, (4) conclusions drawn, (5) method of presentation, and (6) general competence in the subject.

How Much for Research?—It would be extremely difficult to estimate or even "guesstimate" how much time is actually being devoted to research concerned with the oral area. Such research effort can be measured somewhat, however, by learning the amount of money being spent annually for "dental" research. Currently, in the United States, we are spending for research less than 0.5 per cent of the nation's total dental bill, an annual expenditure of one billion 700 million dollars. In the medical area, 3 to 4 per cent of the annual expenditure is being used for research. The pharmaceutical industry devotes 8 to 10 per cent for research. However, the average expenditure for research purposes across the nation, is about 2 per cent of the national product. Most everyone would agree that more funds should be spent for research that concerns the oral area. How much more? One per cent would be 17 million dollars annually. Is this enough? Perhaps, to catch up in this neglected field, as much as 10 per cent of the annual dental expenditure should be allocated to research. (Whether this amount could be spent wisely at this time, would be another question.) Who should provide the money? At present, about 90 per cent of that mere 0.5 per cent is provided by the U. S. Federal government through grants of one type or another. State governments, privately endowed funds, universities, dental societies, and industry pay for the other 10 per cent of the annual research bill. As a matter of national policy, is it appropriate or wise to have the federal government spend this high percentage of the total amount? It might be, especially if this expenditure will motivate the other groups mentioned to provide increasing funds for research purposes. Since dentistry has been so far behind in research, Federal funds might well provide a stimulus to loosen up other funds around the countryside which could be made available for research in the oral area. However, the converse event, leaving it up to the government to provide a higher and higher percentage of the annual research expenditure would be a policy of questionable wisdom. It is apparent that some funds from other sources are available if one but searches them out. For example, the JOURNAL OF DENTAL RESEARCH always has been on the lookout for more funds to expand its size. As the previous page of this issue indicates, Supporting Associates in significant numbers have been found. These groups are both willing and able to provide funds for helping support the JOURNAL, with absolutely no strings attached. This is a relatively small contribution but every bit helps toward the goal of greater support for research.

—FRANK J. ORLAND
EDITOR

AUTORADIOGRAPHS OF SOLDER JOINTS WITH FLUORINE[18]

SAUL M. BIEN AND HERBERT D. AYERS, JR.

Columbia University, New York, N. Y.

PREVIOUS investigation on the nature of the union of solder with rustless alloys indicated that the bond formed as the molten solder wetted the alloy wire.[1, 2] What part the flux plays during soldering is the subject of this study. Flux has been described as an agent which dissolves metallic oxides and keeps the surfaces of the alloys clean during soldering.[3] Flux inclusions have been shown trapped in solder.[4]

One of the problems of the solder point on rustless alloys is crevice corrosion which causes separation of the solder from the rustless alloy.[5-8] It appeared possible that one of the factors in the initiation of crevice corrosion might be the deposition between the solder and the rustless alloy of a film resulting from action of the flux on the metallic oxides, which coats the alloy.[9] Since a fluoride salt is one of the active constituents in most rustless alloy fluxes, fluorine[18] was used as a tracer to follow the fluoride during soldering.

EXPERIMENTAL PROCEDURE

A fluoride flux containing approximately 50 per cent KF, 30 per cent H_3BO_3, 10 per cent $K_2B_4O_7$, and 10 per cent $H_2N.NH_2.H_2O$ with a strongly alkaline pH was used. The fluorine[18] was prepared as an aqueous solution by a (t,n) reaction on oxygen[16] with the irradiation of Li_2CO_3 in a nuclear reactor.[10, 11] This was assayed in a well-scintillation counter which was calibrated against a four pi counter. To 27 ml. carrier-free F^{18} in 1/10 N NaOH was added 15 mg. KF as a carrier and 20 mg. $MgCl_2$. After centrifuging, the supernatant fluid was decanted, leaving a sediment of approximately 1 ml. The sediment containing Mg F^{18}_2 was added to 10 ml. of flux.

An alloy made up of silver 40 per cent, copper 30 per cent, zinc 28 per cent, and nickel 2 per cent (melting point, 1,222° F.) was soldered to 50 sections of 18-8 austenitic steel wrought wire (type 304, diameter 1.3 mm.) and to 10 sections of gold alloy wire (diameter 1.3 mm.) with fluoride flux tagged with fluorine[18] using an air-gas orthodontic blow pipe and air quenched. Ten joints were made as controls with silver-copper-zinc-nickel

This paper was presented in part before the 36th and 37th Annual Meetings of the IADR.

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alloy on 18-8 austenitic steel wrought wire, type 304, using the fluoride flux with no radioisotope added. The wires were washed in detergent for 10 minutes and sandblasted. Each solder joint was then cut below the point of widest contour. Several wires for each mount were counted to determine their relative radioactivity (Table I). Owing to the short half-life it was not feasible to count all wires.

Acrylic resin blocks, 25 mm. in diameter and approximately 14 mm. thick, had been prepared with seven holes. Each hole was 1.3 mm. in diameter

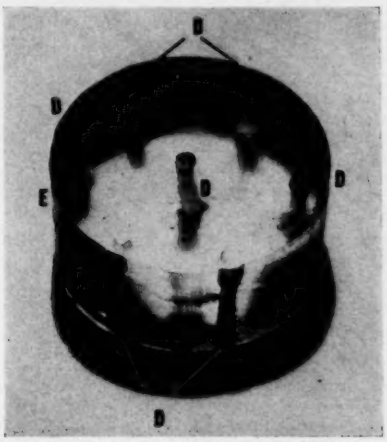

Fig. 1.—Acrylic block with pre-drilled holes into which seven soldered wires *D* were fastened with a hot iron. The holes were eccentrically placed with the top edge notched *E* for orientation in the darkroom.

TABLE I.

RELATIVE RADIOACTIVITY OF SOLDERED SAMPLES

Mount No. 1—*counts/minute*	Mount No. 4—*counts/minute*
579	440
150	114
233	236
167	
264	
Mount No. 2	**Mount No. 5**
222	436
220	824
420	1,206
236	
Mount No. 3	**Mount No. 6**
705	141
109	255
230	118
264	269

	counts/minute
Background	22
Fluxed, soldered, not washed	6,352
Fluxed, soldered, washed, not sanded	268
Fluxed, soldered, washed, sanded	241

and spaced 9 mm. apart. Into each of the holes, a soldered wire was placed and fastened with a hot iron (Fig. 1).

The samples in the acrylic resin mounts were polished on their cross-section with six grades of metallurgical paper (Nos. 160, 240, 320, and 400 carborundum, Nos. 0 and 000 emery). Five mounts, containing seven polished

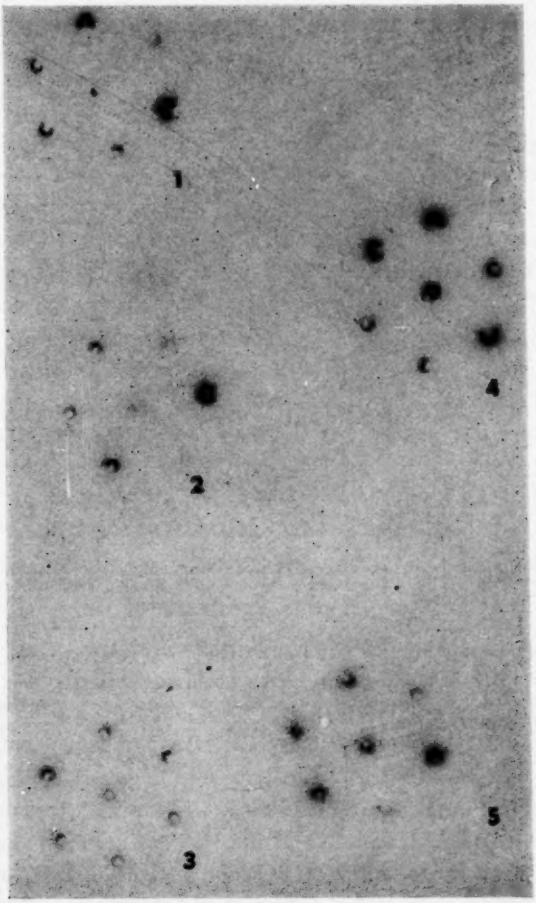

Fig. 2.—Photographic film upon which five acrylic mounts, each containing seven solder joints exposed for infinite half-life, show autoradiographs of varying intensity. The range of relative radioactivity is shown in Table I.

cross-sections each of soldered joints, soldered with flux containing fluorine[18], were placed on Kodak Royal Pan film for 21 hours for exposures of infinite half-life[12] (Fig. 2). Two mounts, containing six polished cross-sections each of joints soldered with flux containing fluorine[18], and one control were placed on separate sheets of Kodak Royal Ortho film. One was removed in 30 minutes; the other in one hour. Five mounts, containing the wires soldered

with flux containing fluorine[18], and controls were placed on a single sheet of film and exposed for 2 hours. The five mounts were again removed and placed on another single sheet of film and exposed for 17 hours.

The silver-copper-zinc-nickel alloy was soldered to cast 65 per cent cobalt–30 per cent chromium–5 per cent molybdenum alloys (diameter 4.0 mm.), using flux containing fluorine[18] for four samples, and control flux for one sample.

Fig. 3.—Solder joints *V* were made on cobalt-chromium-molybdenum samples. Sample *E* was soldered around the straight end of the wire using flux labeled with KF^{18}. Sample *C* was soldered in the curvature using unlabeled flux as a control. Sample *B* was soldered on a straight section away from the end of the wire with flux containing MgF^{18}_2.

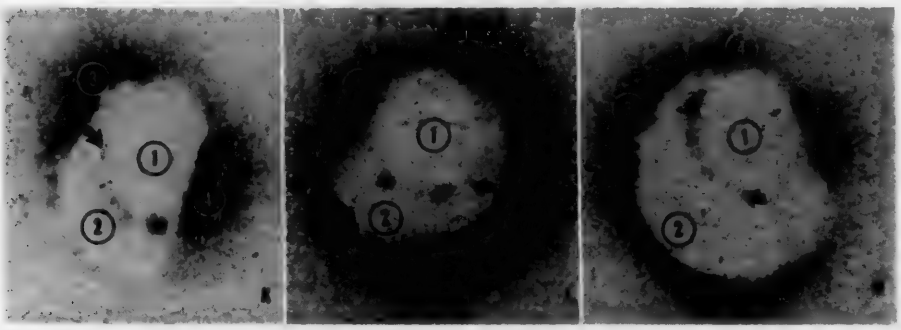

Fig. 4.—The heavy arrows on the autoradiographs of cross-sections of solder joints *K*, *L*, and *M* show the increased radiopacity on the film in contact with the interface of the joint between the austenitic steel wire (*1*) and the silver alloy solder (*2*). A diffuse opacity surrounds the area on the film which was in direct contact with the periphery of the solder (*3*) as well as in areas of the wire to which no solder was added (↓). The specimens were carefully washed and lightly sandblasted prior to being placed in contact with the film.

The soldered cobalt-chrome-molybdenum samples were flattened along their length by grinding on No. 180 carborundum paper, exposing two joints to the film (Fig. 3). All soldered cobalt-chrome-molybdenum samples were placed with the polished sections in contact with a single sheet of film and exposed for 18½ hours. All films were developed for 5 minutes in x-ray developer, washed, fixed, and washed again in the normal manner.

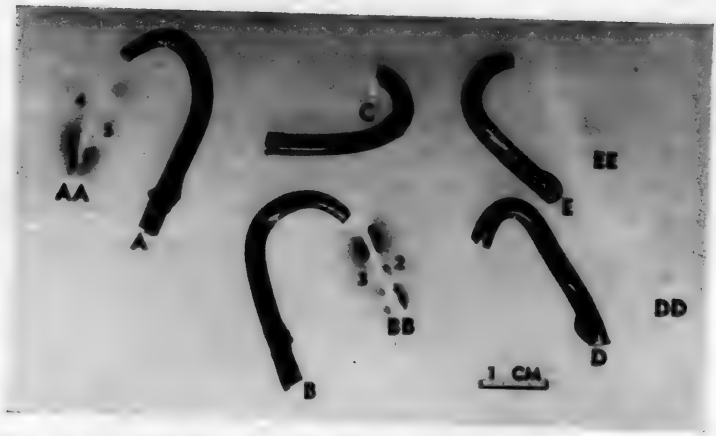

Fig. 5.—*A* and *B* were soldered with flux containing precipitated MgF^{18}_2 and are paired with autoradiographs *AA* and *BB*. Specimen *C* is a control soldered with flux containing no radioisotope. Specimens *D* and *E* were soldered with a flux to which F^{18} was added as a solution and paired with their autoradiographs *DD* and *EE*. Autoradiographs *AA* and *BB*, respectively, are of much greater intensity than autoradiographs *DD* and *EE*. At points *2*, *3*, *4*, and *5* a very fine line of opacity can be traced on the film at the junction between the solder and the Co-Cr-Mo alloy indicating a deposition of F^{18} at the junction. More diffuse opacity is distinctly seen as a halo around the outer surfaces of the solder. Autoradiographs *DD* and *EE* were not sufficiently dense to permit observation of details.

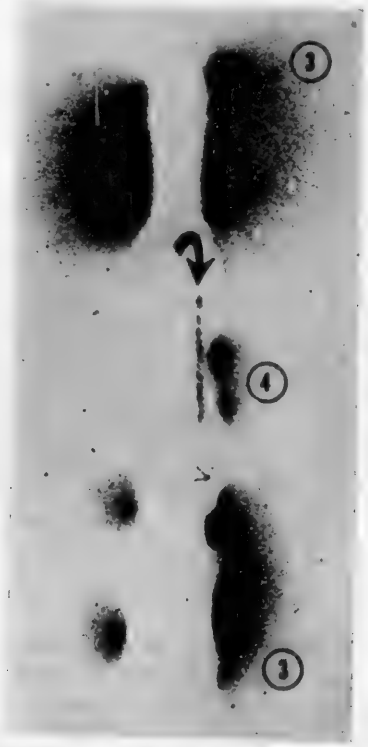

Fig. 6.—An enlargement of autoradiograph *BB* in Fig. 5. Areas (*3*) show a dense opacity on the film in the area of Co-Cr-Mo alloy where flux was applied but no solder. The arrow indicates the interface between the solder and the Co-Cr-Mo alloy. Radiopacity (*4*) delineates the periphery of the solder. The wire was carefully washed and lightly sandblasted after the solder was applied but prior to being placed in contact with the film.

FINDINGS

Autoradiographs of varying intensity appeared on the films. Where 0.95 millicurie in 10 grams of flux was used with the same method, no visible mark on the film appeared. With 1.5 millicuries in 10 grams of flux, barely discernible markings appeared on the film, permitting no detailed examination. On those films which were exposed to the specimens for 30 minutes and one hour, barely discernible markings appeared. On the film which was exposed for 2 hours, markings delineated the periphery of the specimens but there was no visible opacity at a magnification of ×16 between the solder and the wire.

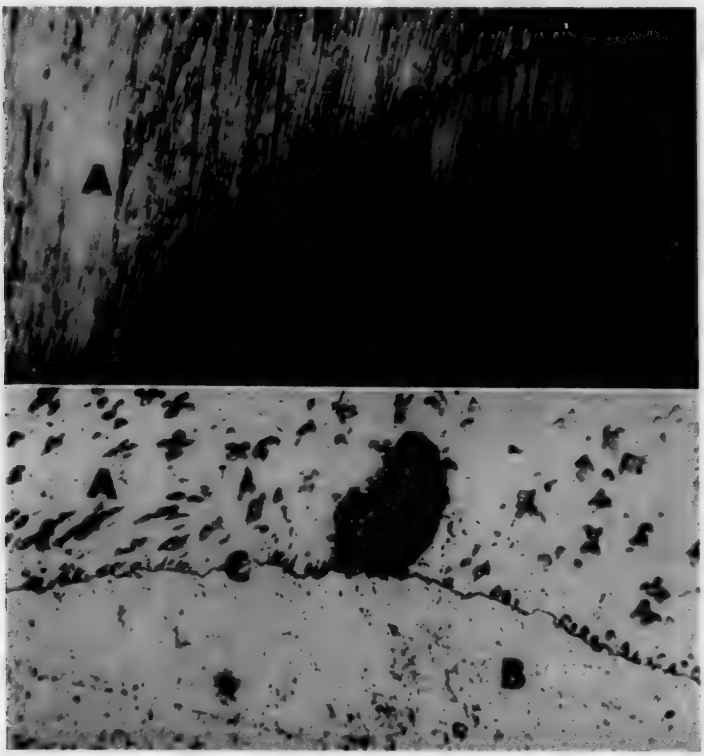

Fig. 7.—A section of the solder joint *K* in Fig. 4 at ×338 (reduced ¼) after exposure to the film. The silver-copper-zinc-nickel alloy *A* is attached to 18-8 austenitic steel (type 304) wrought wire *B* at interface *C*, using flux labeled with MgF^{18}_2. The *upper* photomicrograph shows the surface of the specimen after removal from the autoradiograph. The surface of the specimen in *lower* photomicrograph has been polished and treated with picric acid etch.

Examination of the panchromatic film, exposed for 21 hours to samples soldered with flux containing 3.5 millicuries in 10 grams, showed an aureolus of increased opacity, varying in density from sample to sample around a transparent core measuring 1.3 mm. in diameter. On certain of the autoradiographs, the areas of increased opacity would appear to correspond in shape and area to the solder in contact with the film. On other autoradiographs, at a magnification of ×8, there appeared to be a transparent core,

1.3 mm. in diameter, encircled by a narrow band of increased opacity which was encircled in turn by a transparent zone, which was in turn encircled by an opaque area (Fig. 4).

On 22 of the autoradiographs of the 18-8 austenitic steel, type 304, exposed for 17 hours on orthochromatic film at a magnification of ×8, a thin line of opacity can be seen on the film in positions corresponding to the areas

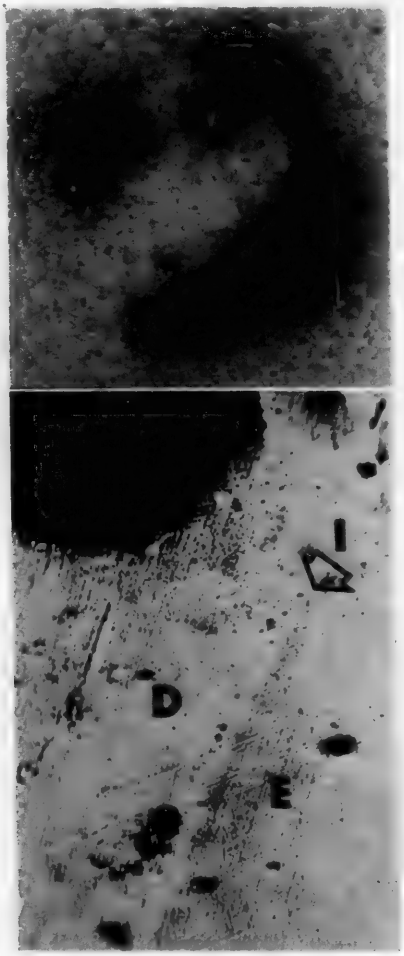

Fig. 8.—Arrow *1* indicates joint between silver alloy solder *D* and wrought gold alloy wire *E*. Vacuolar area *V* on the autoradiograph in the *upper* figure corresponds to *V* on the photomicrograph in the *lower* figure (orig. mag. ×338). There is no trace of opacity corresponding to the joint between the gold and silver alloys on any of the autoradiographs.

of contact of the solder and the austenitic steel with the film as well as an opacity around the entire specimen. Visual examination of the autoradiographs of the cobalt-chromium-molybdenum sections *A* and *B* (Figs. 5 and 6)

shows a similar line of opacity on the film in contact with the solder junction as well as on the periphery of the solder. Radiopacity was also noted on the film in contact with various surfaces of the cobalt-chromium-molybdenum alloy in areas where flux was applied and heated without subsequent soldering.

Microscopic examinations of the soldered sections at ×338 indicate a normal attachment of the solder to the rustless alloy (Fig. 7). On certain of the autoradiographs, in addition to the radiopacity on the periphery of the solder, large areas of opacity appeared on the film in contact with the cross-section of the soldered gold alloy specimens (Fig. 8). Examination of the specimens at ×338 indicates that the areas of opacity on the film were in contact with vacuolar areas in the solder (Fig. 8).

The controls were negative.

SUMMARY

The evidence resulting from the addition of fluorine[18] to the flux indicates that fluorine[18] remains between the solder and the rustless alloy after the joint is completed. Islands of varying concentrations of fluoride salts are deposited at the interface between the rustless alloy and the solder. It would appear likely that crevice corrosion initiates through solution of salts in the area of this deposition.

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THE DISTRIBUTION OF NITROGEN IN HUMAN ENAMEL

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SEVERAL divergent opinions have been stated concerning the concentration of nitrogen in human dental enamel and about changes which may occur in the nitrogen content with advancing age or incidence of caries. One investigator[1] has reported enamel nitrogen concentrations to range between 0.037 and 0.096 per cent while others[2] found values ranging between 0.078 and 0.310 per cent. Mean nitrogen values for pooled enamel have been reported at 0.073 per cent,[3] 0.071 to 0.083 per cent,[4] and 0.057 to 0.064 per cent.[5] These differences may be due, in part, to variations in sampling and/or biologic variations in individual tooth specimens. Large variations have been found in the nitrogen content of enamel from different teeth and from different areas of the same teeth. Determinations of the nitrogen concentration in four successive layers of enamel showed that, in general, outer enamel had a higher nitrogen content than deeper enamel.[6]

Some authors claim that the nitrogen content of enamel increases with advancing age,[1, 2] while others found no significant differences in nitrogen[4] or organic content.[7] No correlation was found between the amount of organic content of sound enamel and the severity of caries[8] or between sound and carious enamel from the same teeth.[6] However, a more recent work,[9] which probably employed more accurate procedures, showed that carious enamel contained 3 to 4 times more organic matter than the adjacent sound enamel. The enamel of deciduous teeth was found to contain significantly more organic matter than that of permanent teeth.[10]

It has been stated that the surfaces of teeth are covered by Nasmyth's membrane and/or acquired plaque,[11] and that the Nasmyth's membrane covering young teeth persists over large areas even in older teeth.[12] The high protein content present at the surface, in the form of a membrane and/or acquired plaque, and at the dentinoenamel junction, in the form of tufts and spindles, has been ignored in many chemical studies because one or both of these areas has been excluded from the samples used for analysis. The object of the present experiments was to determine the distribution of nitrogen in deciduous and permanent teeth from the outermost layer of enamel to the

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inner layer, or dentinoenamel junction. The nitrogen distribution pattern was also related to tooth age. In addition, the nitrogen content of carious surface enamel was investigated.

METHOD AND MATERIALS

Successive layers of enamel were ground from groups of 30 to 50 teeth from the Rochester-Buffalo area. Preliminary investigations showed no significant difference in nitrogen distribution in various tooth types. Therefore, due to the availability and ease in sampling, posterior teeth were generally used. Erupted teeth, grouped according to age (deciduous, under 20, 20-29, 30-49, and over 50), as well as unerupted teeth, were cleaned by brushing with distilled water. Calculus, fillings, and carious material were removed and the teeth, with only sound enamel remaining, were sprayed with distilled water to remove residual debris. The enamel surface area estimated for each tooth group and the weight of the enamel samples were employed to determine the thickness score of each layer by the method of Brudevold and his associates.[13] This permitted a direct comparison of the nitrogen distribution in the tooth group.

Sampling was performed with a motor-driven diamond stone, the ground enamel being collected on a millipore filter by suction. After 6 to 8 thin layers had been ground from the smooth enamel surfaces, the teeth were split into two or more portions with a rotating disk. Adjustments were made in the surface scores to allow for the enamel lost during splitting. Occlusal enamel was then removed and the teeth sprayed as before to remove debris. Three to eight layers were ground from the remaining enamel, the last layer including the dentinoenamel junction material. Two thin layers of white chalky enamel, ground only from initial carious lesions (white spots), were tested for nitrogen concentration.

Purity of enamel samples was determined microscopically, relying on the difference between the refractive indices of the dentin and enamel. Samples containing more than 0.3 per cent dentin were purified by the Manly-Hodge flotation method.[14] During purification, a small percentage of less dense enamel was lost which may contain proportionally more nitrogen than the remaining denser enamel. However, careful sampling generally eliminated the necessity of using this method except for the enamel layers adjacent to the dentinoenamel junction. All samples were transferred quantitatively to weighing bottles and dried for 18 hours in vacuo at 68° C.

Duplicate micro-Kjeldahl determinations, using the digestion mixture of McKenzie and Wallace,[15] were run on 50 mg. samples weighed on a semimicrobalance. Following digestion, which was continued for 20 minutes after clearing, the digestion tubes were assembled, without sample transfer, in the steam distillation apparatus. After a 5-minute distillation period, during which the ammonia was collected in 2 per cent boric acid containing a methyl red, bromcresol green indicator, titrations were done using 0.01 N HCl.

Preliminary standardizations of the apparatus gave a 99.2 per cent return with a 0.34 per cent standard deviation. Pooled samples containing the outer four fifths of the enamel, excluding the cuticle, gave a mean nitrogen value

of 0.080 ± 0.0004 per cent. The pooled enamel contained five dentin particles per 1,000 enamel particles, or a purity of 99.5 per cent. Accurate corrections for dentin contamination are difficult to make because of the varying density, particle size, and nitrogen content of enamel and dentin. However, the corrected value for uncontaminated enamel would be approximately 0.074 per cent nitrogen.

RESULTS

The nitrogen distribution in enamel from all tooth groups followed approximately the same pattern. The concentration was high at the enamel surface with a marked decline in the second layer and a continuing decrease to a minimum at the fifth and eighth layers. In deeper layers, the nitrogen content increased gradually until it reached a high value at the dentinoenamel junction.

The findings are summarized in Tables I and II. In Table I the concentration of nitrogen in the different tooth groups is tabulated by layers of enamel. A wide variation in per cent nitrogen from layer to layer can be noted. In all tooth groups, the nitrogen content in the first layer and the last one or two layers is higher than that of the middle layers.

TABLE I
PER CENT NITROGEN IN SUCCESSIVE LAYERS OF ENAMEL FROM VARIOUS TOOTH GROUPS

ENAMEL LAYER	TOOTH GROUP					
	DECIDUOUS	UNDER 20 YEARS	20-29 YEARS	30-49 YEARS	OVER 50 YEARS	UNERUPTED
1	0.105	0.104	0.091	0.088	0.148	0.103
2	0.096	0.054	0.069	0.066	0.076	0.059
3	0.077	0.027	—	0.069	0.058	0.066
4	0.079	0.024	0.045	0.042	0.044	0.051
5	—	0.041	0.070	0.051	0.055	0.053
6	0.066	—	0.077	0.064	0.054	0.043
7	0.088	0.059	0.082	0.055	0.047	0.084
8	0.090	0.064	0.043	0.044	0.034	—
9	0.194	0.105	0.077	0.052	0.038	0.082
10	—	0.103	0.082	—	0.042	0.085
11	—	0.139	0.061	0.058	0.036	0.068
12	—	—	0.066	0.064	0.044	0.072
13	—	—	0.146	0.082	0.209	0.099
14	—	—	—	0.063	—	—
15	—	—	—	0.106	—	—

Table II gives the nitrogen concentration of each tooth group in the following portions of enamel: (1) surface enamel (first layer), (2) outer 20 per cent, excluding the surface enamel, (3) middle portion, (4) inner 20 per cent, excluding the junction enamel, (5) junction enamel, and (6) total enamel. The nitrogen content of surface enamel in all tooth groups, with the exception of the over 50 group, falls between 0.088 to 0.105 per cent. The oldest teeth have considerably more nitrogen in the surface enamel (0.148 per cent). In all, except the over 50 and deciduous groups, minimal concentrations of nitrogen are found in the subsurface enamel (outer 20 per cent) and a slight increase occurs in the middle enamel. In contrast to the other

groups, the teeth over 50 years of age and deciduous teeth appear to contain the least nitrogen at the latter enamel depth. In all teeth, except the unerupted ones, the nitrogen concentration reaches a maximum at the dentinoenamel junction. Here, as at the surface, enamel from the over 50 year group is considerably higher in nitrogen content than that of other permanent teeth. Unerupted teeth have a slightly lower nitrogen concentration in enamel nearest the junction than in surface enamel. Nitrogen in total enamel of all tooth groups varies within a narrow range from 0.064 to 0.078 per cent, except for deciduous enamel which contains a significantly higher nitrogen concentration than permanent enamel. The mean nitrogen concentration for all the enamel portions, included in the present study, is 0.075 per cent and, excluding deciduous teeth, the mean nitrogen value for enamel from permanent teeth is 0.070 per cent.

TABLE II
AVERAGE NITROGEN CONTENT OF ENAMEL PORTIONS FROM VARIOUS TOOTH GROUPS

TOOTH GROUP	SURFACE ENAMEL	OUTER 20% ENAMEL	MIDDLE ENAMEL	INNER 20% ENAMEL	DENTINO-ENAMEL JUNCTION	TOTAL ENAMEL
Deciduous	0.105	0.096	0.089	—	0.194	0.099
Under 20 yr.	0.104	0.036	0.076	0.103	0.139	0.072
20-29 yr.	0.091	0.069	0.079	0.064	0.146	0.078
30-49 yr.	0.088	0.055	0.072	—	0.106	0.064
Over 50 yr.	0.148	0.058	0.040	0.044	0.209	0.068
Unerupted	0.103	0.062	0.058	0.075	0.099	0.070
Total average	0.106	0.062	0.069	0.072	0.148	0.075
Excluding deciduous teeth	0.106	0.056	0.065	0.072	0.140	0.070

A higher average nitrogen content in deciduous than in permanent teeth is illustrated in Fig. 1. The relationship of tooth age of permanent erupted teeth to nitrogen distribution is shown in Fig. 2. In all groups studied, the nitrogen distribution pattern is approximately the same, that is, high at the enamel surface with a decline in the subsurface or middle enamel and an increase in the deeper enamel to a high nitrogen value at the dentinoenamel junction. It will be seen from Fig. 2 that there is no consistent change in the concentration of nitrogen in enamel with age. However, differences in the distribution of nitrogen do appear among the tooth groups. In the over 50 year teeth, the minimal level of nitrogen extends farther into the enamel than in the younger teeth. In addition, the graph illustrates the higher nitrogen concentration in surface and junction enamel of the over 50 year teeth. These findings were duplicated in another batch of over 50 year old teeth. In the 20 to 29 year group, greater differences are found in the nitrogen concentrations of succeeding layers than in the other tooth groups. No reason can be given for the apparently inconsistent results in this group.

Analysis of carious enamel and noncarious enamel from pooled permanent, erupted teeth showed carious enamel (0.140 per cent) to contain twice as much nitrogen as noncarious enamel (0.070 per cent).

DISCUSSION

From our results it is evident that all enamel tends to follow the same nitrogen distribution pattern, with the greatest concentration at the external surface and at the dentinoenamel junction. High nitrogen content at the

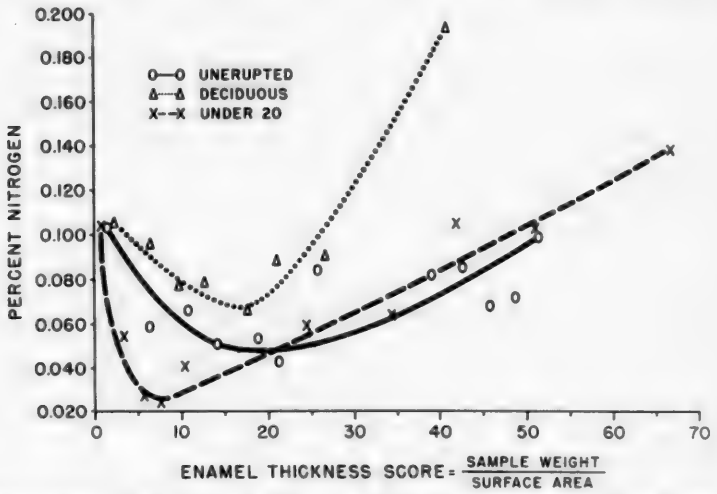

Fig. 1.—Comparison of the enamel nitrogen distribution from the enamel surface to the dentinoenamel junction in deciduous and permanent teeth.

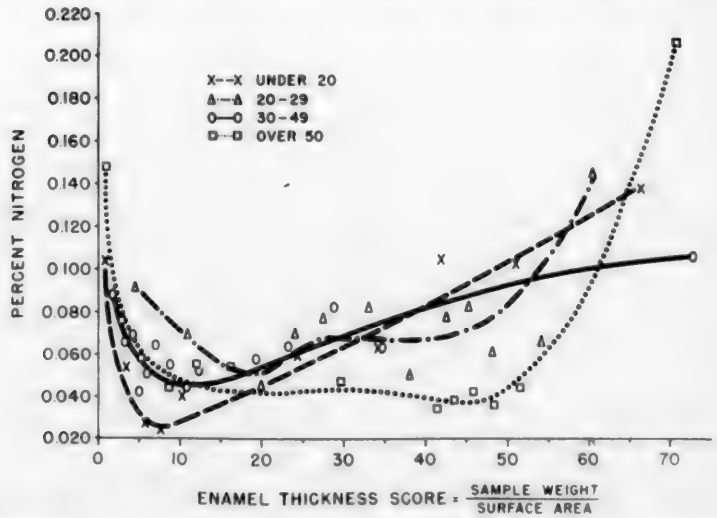

Fig. 2.—Nitrogen distribution from the enamel surface to the dentinoenamel junction in erupted permanent teeth of various ages.

enamel surface must be related to the presence of Nasmyth's membrane and/or a layer of organic deposition. As the dentinoenamel junction is approached, the organic constituents of enamel are undoubtedly increased by the projection of tufts and spindles.

The findings of some investigators of no change in the concentration of enamel nitrogen with age are supported by our work only as far as total enamel is concerned. The differences observed in the oldest compared to younger tooth groups, i.e., an increase in nitrogen concentration in the outer and innermost enamel and a concomitant decrease in the middle portion, undoubtedly reflect changes resulting from prolonged exposure to the oral environment.

An increase in nitrogen in surface enamel of old teeth is unlikely to be due to external deposition of organic material, since all the teeth were thoroughly cleaned prior to sampling. It is rather believed that the presence in old teeth of numerous surface defects, produced by trauma or partial decalcification, permits impregnation of salivary organic material. That partial decalcification of surface enamel can take place is suggested by the finding of a gradual loss of carbonate from the outermost portion of the enamel with age.[16] The organic material deposited in the outermost surface enamel apparently does not calcify to any extent, possibly because of the varying pH at the tooth surface. Hence the increased nitrogen concentration.

The decrease of nitrogen in the middle portion of enamel of old teeth is believed to be associated with the many cracks present in these teeth. According to Sognnaes, cracks are synonymous with lamellae, that is, they will fill in with organic material and eventually calcify.[17] The fact that powdered enamel suspended in saliva readily removes calcium and phosphate[18] suggests that similar reactions may occur in the cracks. It is likely that the enamel adjacent to the cracks will acquire calcium salts from saliva and undergo further calcification. A progressive calcification of this type has been observed in hydroxyapatite exposed to calcifying solutions[19] and may be generally expected in incompletely calcified material which is in contact with solutions containing high concentrations of calcium and phosphate at approximately neutral pH. Thus, cracks may act as separating columns in taking up ions and gradually deprive the saliva of mineral as it approaches the dentinoenamel junction. As a result, more organic than inorganic material may deposit in the inner portion of the enamel as is, in fact, suggested by the increase of nitrogen in enamel adjacent to the dentin in old compared to young teeth. Other factors may contribute to this latter effect. For example, it is not known to what extent soluble peptides and proteins present in the enamel[20] may diffuse in from the pulp via the dentin. Whatever factors are involved in the complex changes associated with age, it is significant that the bulk of the enamel tends to consolidate or increase in calcification, and that aging therefore seems to strengthen rather than weaken this tissue.

The finding that enamel of deciduous teeth has more nitrogen than that of permanent teeth supports a similar observation by Stack.[10] Evidently deciduous enamel is not as well calcified as permanent enamel.

A higher organic content in carious than in noncarious enamel was found by Stack,[9] as well as by the present authors, suggesting partial decalcification as a result of the carious process and the possible infusion of organic material.

SUMMARY

1. There is a definite nitrogen distribution pattern throughout all dental enamel, with the greatest amounts of nitrogen at the surface and dentinoenamel junction.

2. There appears to be a difference in nitrogen distribution of permanent teeth with relation to age. In the over 50 age group, the nitrogen content of the surface and junction enamel is comparatively higher and the minimal enamel nitrogen content occurs at a greater enamel depth than in younger teeth.

3. No significant difference occurs in the total nitrogen content of enamel of permanent teeth with advancing age.

4. Deciduous enamel contains more nitrogen than permanent enamel.

5. Carious enamel contains approximately twice as much nitrogen as non-carious enamel on a per cent/weight basis.

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DISTRIBUTION OF SOME ENZYMES IN SOFT TISSUES OF THE ORAL CAVITY

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MOST authors,[1-5] in examining the phosphatase and esterase activity of oral tissues, have dealt with the enzyme content of individual tissues. We have studied the alkaline phosphatase, acid phosphatase and nonspecific esterase activities in various normal and some pathologic oral tissues. We hope to present an over-all picture of the localization of these enzyme activities in the normal oral cavity before correlating any changes observed with pathologic processes.

MATERIALS AND METHODS

The following oral biopsy specimens were studied (the number of specimens studied is listed in brackets in each case): (1) normal oral mucosa [26], (2) squamous cell carcinoma of lower lip [5], tongue [2], floor of mouth [1], and buccal mucosa [1], (3) leukoplakia [17], (4) capillary hemangioma [3], cavernous hemangioma [2], (5) papilloma of tongue [2], and lower lip [1], (6) myoblastoma of tongue [1], (7) hard fibroma [3], ossifying fibroma [1], (8) fibrous epulis [2], granulomatous epulis [2], giant cell epulis [4], (9) inflammatory hyperplasia of the mucous membrane with epithelial parakeratosis [5], (10) nonspecific ulcer of lower lip [3], and (11) adenoma of palate [1].

Blocks of tissue were fixed and dehydrated in cooled acetone for 24 hours, treated with benzene for three 15-minute periods, and embedded in paraffin within 1 hour. The temperature of the paraffin did not exceed 56° C. Sections at 7 to 10 μ were cut from the paraffin blocks. In some cases, excised tissue was fixed in cooled formol for 4 to 12 hours and frozen sections at 15 μ were cut from the frozen tissue blocks.

The following methods were used for demonstration of localization of the enzyme activities: (1) alkaline phosphatase—(a) Gomori's technic[6] and (b) azo-coupling method, modified by Lojda[7, 8]; (2) acid phosphatase—Gomori's technic[6] and azo-coupling method of Lojda; and (3) nonspecific esterase—azo-coupling method of Pearse[9] and Lojda.[7, 8]

Control sections were incubated in media containing no substrate. For inactivation tests, Lugol solution was used for phosphatase inactivation, 0.01 M silver nitrate and 0.01, 0.001, and 0.0001 M eserine salicylate for esterase

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TABLE I

ENZYME LOCALIZATION IN SOFT TISSUES OF ORAL CAVITY

ENZYME	EPITHELIUM OF THE MUCOUS MEMBRANE									
	WITHOUT KERATINIZATION			WITH INCOMPLETE KERATINIZATION			WITH KERATINIZATION OF THE EPIDERMAL TYPE			
	BASAL LAYER	STRATUM SPINOSUM	SUPERFICIAL LAYER	BASAL LAYER	STRATUM SPINOSUM	SUPERFICIAL LAYER	BASAL LAYER	STRATUM SPINOSUM	STRATUM GRANULOSUM	STRATUM CORNEUM
Alkaline phosphatase	–	–	–	–	–	In some places +	–	–	Sometimes +	In deeper layers sometimes +
Acid phosphatase	±	±	±	±	±	∓	±	±	Sometimes +	∓
Nonspecific esterase	+	++	In some places +	∓	+	++	∓	+	+	In deeper layers +++

ENZYME	PROPRIA MUCOSAE			SUBMUCOSA				MUSCLES	
		CONNECTIVE TISSUE			SALIVARY GLANDS				
	VESSELS	CELLS	FIBERS	VESSELS	SEROUS ACINI	MUCINOUS ACINI	DUCTS	VESSELS	MUSCLE FIBERS
Alkaline phosphatase	Endothelium of capillaries +++ Arterioles +	Some cells of infiltrate +	–	Endothelium of capillaries +++ Arterioles +	Sometimes +	Sometimes +	–	Endothelium ++	–
Acid phosphatase	–	Some cells of infiltrate +	–	–	Sometimes +	Sometimes ±	–	–	–
Nonspecific esterase	Pericytes +++	Fibrocytes and some cells of infiltrate ++	–	–	Sometimes + Basket cells +	Sometimes ±	+++	–	++

TABLE II
ENZYME LOCALIZATION IN SKIN OF LIP

ENZYME	SKIN					
	EPIDERMIS				CORIUM	
	BASAL LAYER	STRATUM SPINOSUM	STRATUM GRANULOSUM	STRATUM CORNEUM	PAPILLARY LAYER	RETICULAR LAYER
Alkaline phosphatase	–	–	Sometimes +	Sometimes in lower parts +	Endothelium of capillaries +++ and arterioles +	Endothelium of capillaries +++ and arterioles +
Acid phosphatase	+	+	Sometimes +	Sometimes +	–	–
Nonspecific esterase	+	+	+	In deeper layers ++	Fibrocytes and capillary pericytes ++	–

ENZYME	ADNEXA			
	SEBACEOUS GLANDS	SWEAT GLANDS		HAIR AND HAIR FOLLICLE
		SECRETORY PART	DUCT	
Alkaline phosphatase	–	+	–	Papilla and fibrous sheath of bulbus +++
Acid phosphatase	+	+	+	Bulb, hair cortex, and inner epithelial sheath +
Nonspecific esterase	Periphery + Center and transition region ++	Secretory cells ++ Myoepithelial cells +	Cells adjacent to lumen ++	Cortex ± Inner and outer epithelial sheath ++

inactivation. The sections were incubated in these media for one hour while controls were incubated in saline. Afterward azo-coupling was performed. Parallel sections were stained with hematoxylin and eosin.

RESULTS

Enzyme localization was found to be the same in paraffin sections after acetone fixation or in frozen sections after formol fixation. The intensity of the reaction was somewhat weaker in the paraffin sections.

Enzymatic distribution in normal oral mucosa and underlying soft tissues is shown in Table I. For comparison, enzymatic distribution in the skin and its appendages is shown in Table II.

In *pathologic processes,* the results were found to differ from the controls in the following cases.

Squamous cell carcinoma of the floor of the mouth (one case, highly anaplastic tumor). Intensive nonspecific esterase activity was found in all the neoplastic cells (see Figs. 11 and 12).

Giant-cell epulis: Alkaline phosphatase activity was found in giant cells, fibrocytes, and fibroblasts. In some of the giant cells, and in the fibrocytes, nonspecific esterase activity was seen as well.

Fibrous epulis: Alkaline phosphatase activity was found in connective tissue fibers with both of the described methods.

Hemangioma: Alkaline phosphatase activity in the vessels of these tumors was much lower than the activity in the vessels of normal tissue. Sometimes no phosphatase activity could be detected at all. Newly formed blood vessels in granulation tissue show a similar, very low and, sometimes negative, alkaline phosphatase reaction.

Adenoma of palate: The alkaline phosphatase activity was present in some ducts, but esterase activity was completely absent.

DISCUSSION

A list of the enzymatic localization in soft tissues of the mouth follows.

1. *Alkaline phosphatase activity* was detected in the following structures.

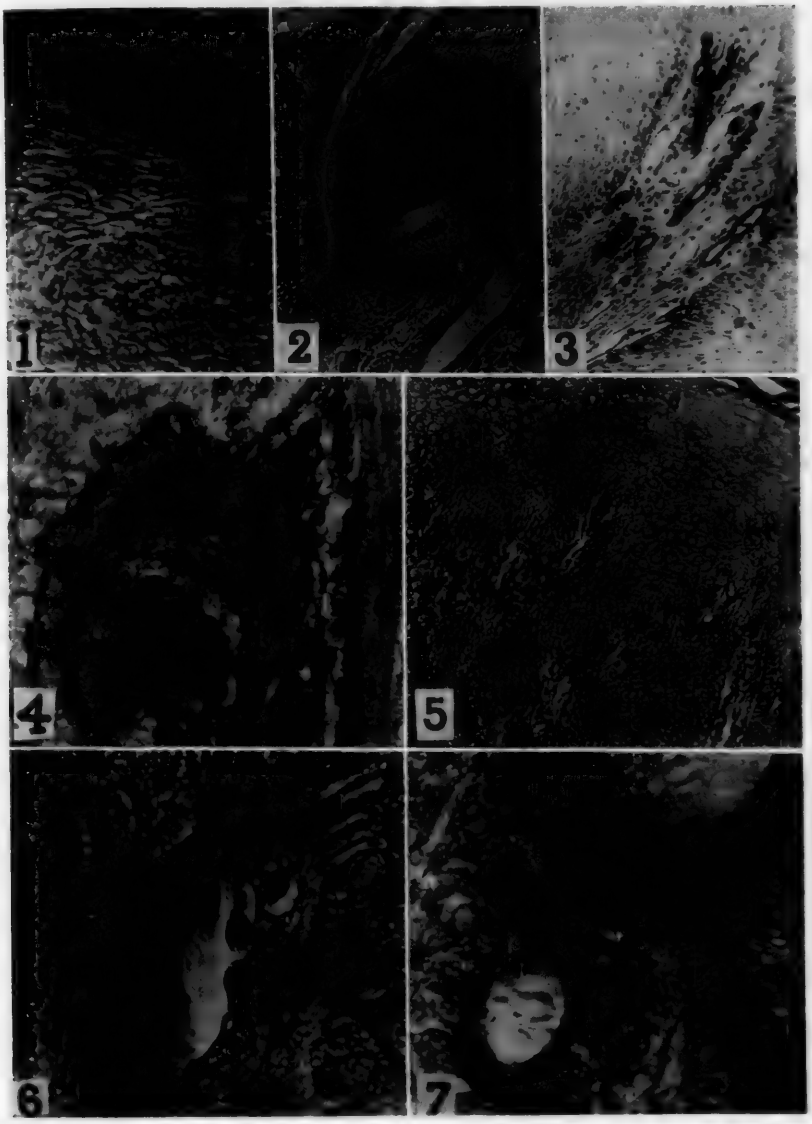
a. Endothelial cells of mature capillaries. Activity in mature vessels was high, while it was lower in arterioles, and very low or absent in immature blood vessels of granulation tissue and hemangiomas.

b. Some cells of the inflammatory infiltrate, notably leukocytes.

c. Osteoblasts, osteoclasts, osteocytes and, to a lesser extent, in fibrocytes and fibroblasts of ossifying fibromas.

d. Giant cells, fibroblasts, and fibrocytes of giant-cell epulides. The activity of connective tissue fibers seen in fibrous epulides was probably due to a diffiusion of the enzyme.

e. Epithelial cells of the stratum granulosum and adjacent layer of the stratum corneum (Fig. 1). This reaction was not found consistently, even when both methods were employed. The reaction may be a residual activity



Figs. 1-7.—AP: paraffin section after acetone fixation. DFB: diazoniumfluoroborate. TFB: tetrazoniumfluoroborate.

Fig. 1.—Alkaline phosphatase in stratum granulosum and in lower layers of stratum corneum of lip epidermis. AP, α-naphthylphosphate, DFB of 5-chloro-2-toluidine (×500).

Fig. 2.—Alkaline phosphatase in hair papilla and in the part of fibrous sheath surrounding bulb of the hair follicle of the lip. AP, α-naphthylphosphate, DFB of 5-chloro-2-toluidine (×70).

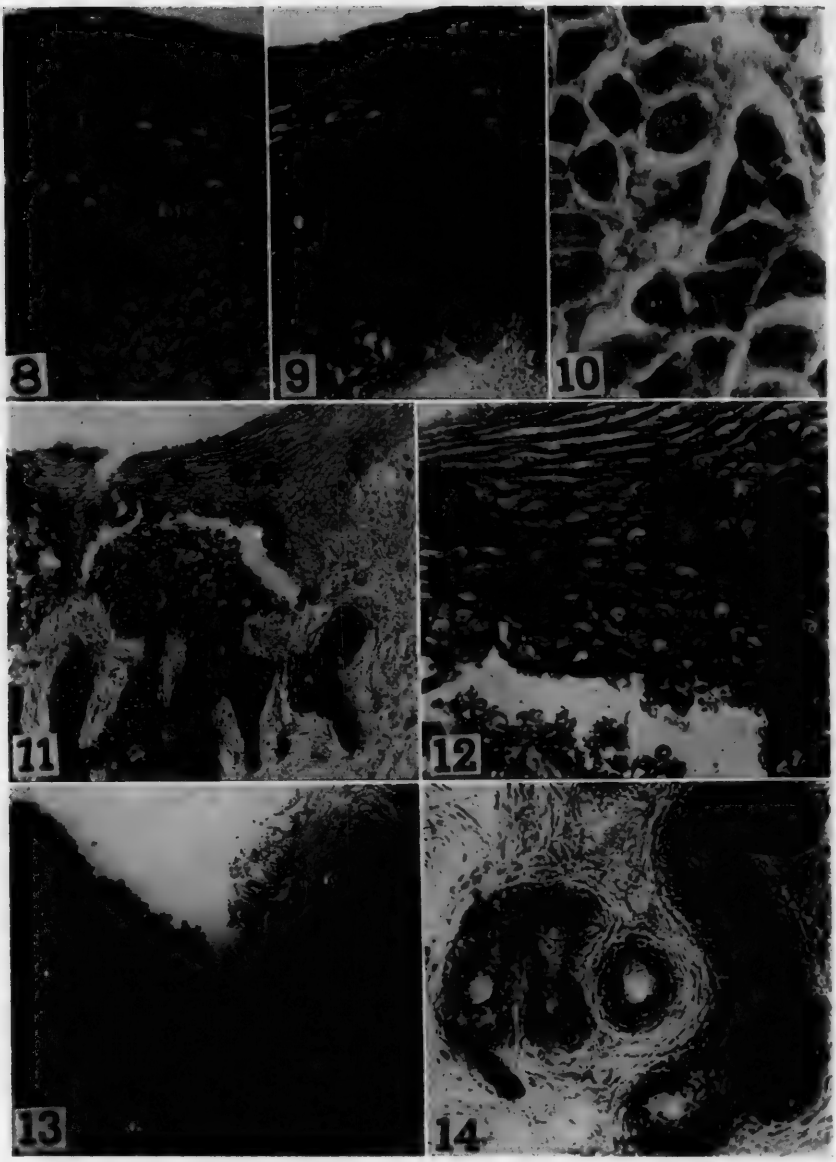
Fig. 3.—Alkaline phosphatase in vessels in connective tissue of gingiva. AP, Gomori's reaction, Kernechtrot (×80).

Fig. 4.—Alkaline phosphatase in osteoclasts, osteoblasts, and some osteocytes of ossifying fibroma. AP, α-naphthylphosphate, DFB of 4-chloro-2 anisidine (×250).

Fig. 5.—Nonspecific esterase in pericapillary fibrocytes of propria of buccal mucosa in inflammatory hyperplasia. AP, α-naphthylacetate, TFB of o-dianisidine (×90).

Fig. 6.—Detail from preceding section (×800). Compare Fig. 3.

Fig. 7.—Nonspecific esterase in duct cells of salivary gland of lower lip. AP, α-naphthylacetate, DFB of 5-chloro-2-toluidine (×700).



Figs. 8-14.—AP: paraffin section after acetone fixation. DFB: diazoniumfluoroborate. TBF: tetrazoniumfluoroborate.

Fig. 8.—Nonspecific esterase in epithelium with incomplete keratinization; marked reaction in superficial layers. AP, α-naphthylacetate, TFB of o-dianisidine (×300).

Fig. 9.—Corresponding section after cooled formol fixation. The reaction is stronger and it appears in stratum malpighii as well. However, in superficial layers, it is much more intense (×210).

Fig. 10.—Nonspecific esterase of striated muscle fibers of tongue. AP, α-naphthylacetate, TFB of 5-chloro-2-toluidine (×20).

Fig. 11.—Nonspecific esterase in spinocellular carcinoma of the floor of the mouth. AP α-naphthylacetate, TFB of o-dianisidine. There is a marked reaction in the tumor epithelium. Note islets of positively reacting cells in epithelium of mucous membrane (×80).

Fig. 12.—Detail from preceding figure clearly showing positive reacting islets (×400).

Fig. 13.—Nonspecific esterase in leukoplakia (right) of the floor of the mouth; normal epithelium (left). AP, α-naphthylacetate, TFB of o-dianisidine (×80).

Fig. 14.—Nonspecific esterase in sweat gland of lip. AP, α-naphthylacetate, TFB of o-dianisidine (×200).

of acid phosphatase as described by Spier and Martin.[10] Cabrini and Carranza[3] described a positive reaction of alkaline phosphatase in the epithelial cells covering the top of the papillae of the gingival mucous membrane. They used Gomori's reaction with nucleic acid as substrate. However, diffusion of calcium phosphate from very highly active capillaries must be eliminated.

f. Secretory cells of salivary glands; inconsistent reaction.

g. Skin appendages. This finding is in accordance with the findings of Spier and Martin,[10] Montagna and Ellis[11] and other authors.

2. *Acid phosphatase activity* was localized in (a) all layers of keratinizing epithelium except the stratum corneum (epithelium with parakeratosis showed positive reaction in the surface layer as well), (b) cells of inflammatory infiltrate, notably leukocytes, and (c) serous acini of salivary glands; these acini showed a greater reaction than did mucous acini, but neither reaction was extensive.

3. *Nonspecific esterase activity* was found in the following structures.

a. Stratum malpighii of nonkeratinizing epithelium. Where keratinization occurred, transfer of the enzyme activity to surface layers or its disappearance in deeper layers was observed in paraffin sections (Figs. 8, 9, 13). An intensely positive reaction was found in acanthotic pegs and cells of an anaplastic squamous cell carcinoma of the floor of the mouth. Nonspecific esterase activity in acanthotic pegs of psoriasis was described by Braun-Falco.[12]

b. Fibrocytes of connective tissue generally, and pericytes of capillaries in the papillary layer of the skin (Figs. 5, 6).

c. Some cells of inflammatory infiltrate.

d. Salivary glands; activity was generally low and, if any was detected at all, it was greater in serous cells than in mucous. The cells of duct epithelium consistently showed a marked positive reaction. Burstone[1, 2] described similar results.

e. Striated muscle fibers in sarcoplasma beneath sarcolemma (Fig. 10); sometimes activity of a lower degree was found between myofibrils as well.

f. Skin and its appendages (Fig. 14); our results here do not differ materially from those described by Findlay,[13] Montagna[14] and Braun-Falco.[12]

Nonspecific esterase activity in all localizations was inhibited by 0.01 M silver nitrate. The activity of this enzyme in striated muscle and in salivary glands was inhibited by 0.01 M eserine salicylate as well. This inhibition was less marked in the salivary gland tissue. Lower concentrations of the eserine inhibition had no effect on esterase activity.

SUMMARY

An over-all picture of the distribution of alkaline phosphatase, acid phosphatase, and nonspecific esterase activities in soft tissues of the oral cavity, under normal and some pathologic conditions, is presented.

There appears to be no constant picture of enzyme activity localization of substantial diagnostic significance. The activity of those enzymes studied

here, especially that of nonspecific esterase, probably changes with the degree of normally occurring keratinization and other dyskeratotic processes in the epithelium. Further studies of this problem are needed.

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DENTAL CONDITIONS OF PRESCHOOL CHILDREN (ONE TO SIX YEARS OF AGE) IN SZEGED, HUNGARY

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MOST reports on the prevalence of dental caries are restricted to examinees attending compulsory schools. The caries data of the 6- to 14-year-old children reveal that at the time of school admission, dental decay had progressed in the deciduous dentition to a remarkable degree.

We have, however, very limited data concerning the prevalence of caries in preschool age children in Hungary. According to Oravecz,[1] in 1937, carious lesions were noted in 30 per cent of examinees 3 years of age and increased to 80 per cent in the sixth year. Bruszt[2, 3] examined 26 persons from 3 to 5 years of age and recorded a heavy prevalence of caries in the deciduous dentition. At Nagybaracska, in spite of a rather low prevalence of decay in the permanent teeth, an average of 1.2 carious and filled deciduous teeth was reported among the 3-year-old children.

It seemed necessary to us to extend our study on the prevalence of dental caries in Szeged and the surrounding area to children of preschool age. Thus, we are concerned with completing our own data and contemporaneously we hope to furnish some basic information upon the caries of this hitherto neglected age group.

We extended these examinations to the most juvenile group in which examinations were feasible; so, in our material, 206 children in their second year of life are included.

MATERIALS AND METHODS

Examinations were carried out clinically by natural sunlight with mirror and probe. All discolored pits and fissures in which the point of the probe was caught were regarded as carious. Defects of enamel and dentin were counted as circular caries, as well as chalky discoloration at the neck of the anterior teeth which involved at least half and usually the whole circumference.

The next birthday is referred to as the age of the child. In this way we are able to compare our findings with those of Fulton.[4]

Missing deciduous teeth were disregarded.

Examinations were carried out in the infants' homes and kindergartens during the second half of 1957. Children entering public school in September

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were examined in August, 1957 (public school can be entered in Hungary by children who have passed their sixth birthday prior to September 1 of that year). Altogether 2,699 children of both sexes were examined dentally; 1,411 boys and 1,288 girls. The distribution into the different age groups and the two sexes is summarized in Table I.

TABLE I
NUMBER OF EXAMINEES IN THE SINGLE YEARS, SEPARATED ACCORDING TO SEX

	NEXT BIRTHDAY						TOTAL
	2	3	4	5	6	7	
♂	96	111	244	264	305	391	1,411
♀	110	89	217	255	248	369	1,288
Totals	206	200	461	519	553	760	2,699

A sufficient number of children was examined, even in the youngest age group, to regard our data as representative for preschool children in Szeged.

RESULTS

A. The Deciduous Dentition.—

1. *df and MDF counts in children of 2 to 7 years:* No conspicuous difference was observed between boys and girls, in regard to the df count. In some groups, more caries prevailed in boys, in others, in girls.

TABLE II
df AND MDF COUNTS IN CHILDREN OF 2 TO 7 YEARS

	NEXT BIRTHDAY						TOTAL
	2	3	4	5	6	7	
df Count							
♂	0.17	0.85	1.86	3.32	4.58	5.15	3.44
♀	0.12	0.69	2.13	3.06	4.19	4.52	3.13
DMF Count							
♂	-	-	-	0.01	0.02	0.13	
♀	-	-	-	-	0.06	0.23	
Totals							
df	0.15	0.78	1.99	3.19	4.40	4.85	3.29
DMF				0.005	0.03	0.18	

Caries were first seen in children of 2 years of age, prior to the completion of the deciduous dentition. The df count amounts to 0.15 at 2 years, and displays a steady increase with advancing age. This increase is rather considerable up to 6 years, therafter the df curve becomes somewhat flattened (Fig. 3, *df*). This finding is indicative of the fact that, in school entrants, the first great carious attack has been completed. After 6 years of age, apparently no new tooth units in the deciduous dentition are attacked by caries, but rather there is a progress of decay in the carious teeth.

Between 2 and 3 years, the df count displays a fivefold increase (from 0.15 to 0.78); between 3 and 4 years, the increase is only two and one-half times (from 0.78 to 1.99). The absolute increase is, however, more marked from 3 to

4 years than from 2 to 3 years. This is apparent also from Fig. 3, which displays a steeper increase between 3 and 4 years than formerly. From 3 to 6 years of age, a nearly constant increase is displayed.

2. *Caries resistance in the deciduous dentition* (Table III): According to available literature, eruption of the deciduous dentition starts at 6 months of age. We were able to find carious lesions in 5 per cent of children having passed their first birthday. It may be assumed that dental caries starts immediately after the eruption of teeth. Fulton[4] found no carious teeth in children 6 months of age. This finding is not contradictory to the aforementioned assumption. By increasing the number of examinees, quite surely a few would be found with carious teeth at 6 months of age.

TABLE III
PERCENTAGE OF CARIES-FREE INDIVIDUALS IN THE DIFFERENT YEARS

	NEXT BIRTHDAY					
	2	3	4	5	6	7
♂	94.8	75.6	52.8	29.1	17.0	7.6
♀	95.4	74.1	47.9	31.0	18.5	10.2
Totals	95.0	75.0	50.0	30.0	17.9	8.6

Seventy-five per cent of examinees 3 years of age are still free of caries. Whereafter, a rapid decrease is found in the frequency of caries-free individuals. At 4 years, the frequency amounts to 50 per cent, before entering school only to 8.6 per cent.

No difference was seen between the two sexes as regards freedom from caries.

3. *The components of the df count* (Table IV): No consideration was given to missing (extracted) deciduous teeth since we were not able to obtain reliable information from the examinees hereupon. Our group consists of persons who had not yet reached the exfoliation age of deciduous teeth. Nevertheless, we suppose that delayed eruption might have caused some distortion of the picture with 2- and 3-year-old children, and perhaps precocious exfoliation with 5-year-olds. Missing deciduous teeth are, thus, not necessarily sequelae of caries.

From Table IV, two statements are to be emphasized. First: In children of 2 years of age some teeth were so completely destroyed that only root remnants were found in the mouth. At this age, caries of anterior teeth amounts to 60 per cent of the total caries experience. Roots were found at 2 years of age in the anterior teeth only, but at 3 years roots of deciduous molars were also observed. Beginning at the fourth year, a marked increase occurs in the number of root remnants present.

TABLE IV
THE COMPONENTS OF THE df COUNT (PERCENTAGE DISTRIBUTION)

	NEXT BIRTHDAY						TOTAL
	2	3	4	5	6	7	
Roots	3.2	2.9	9.8	8.2	16.9	16.8	14.0
Fillings	–	–	1.9	2.8	3.8	4.9	2.8
Caries	96.8	97.1	88.3	89.0	79.3	78.3	83.2

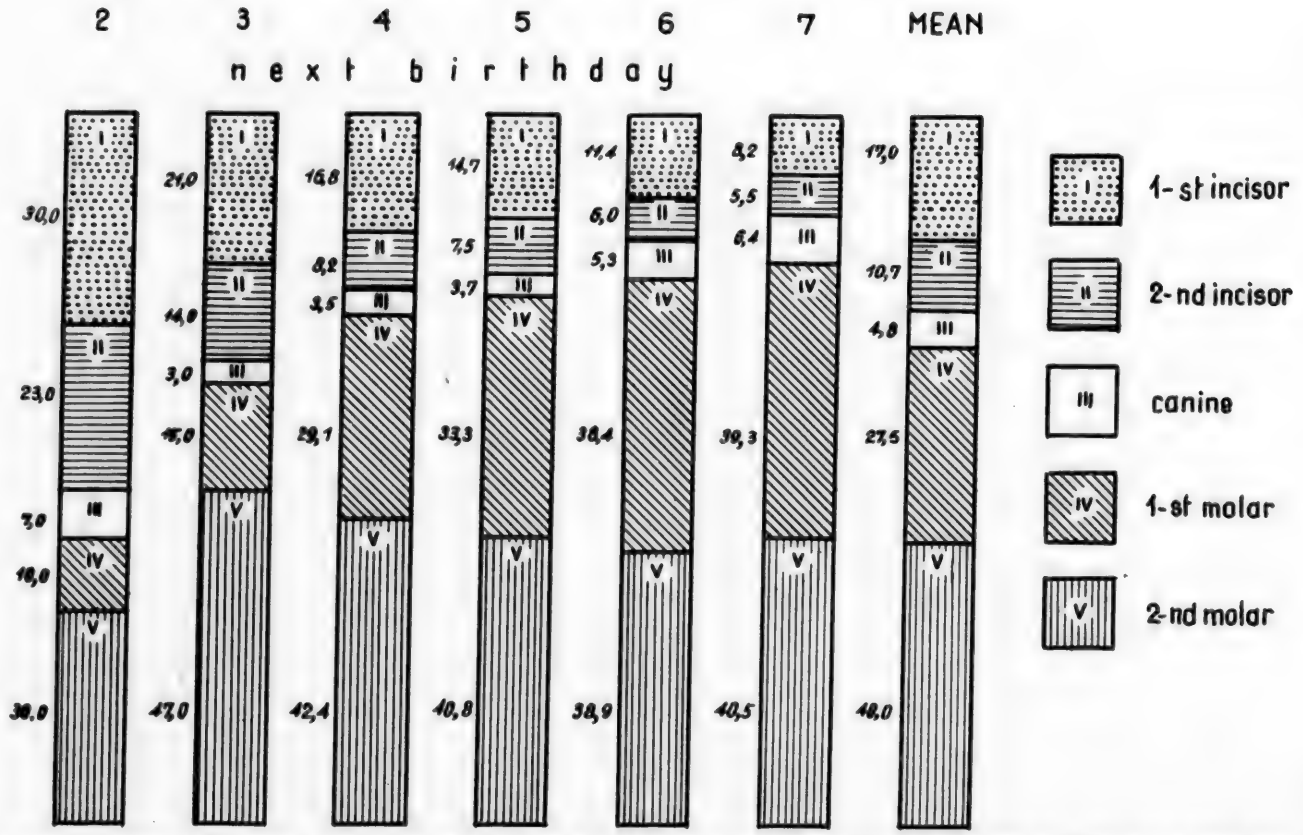

Fig. 1.—Prevalence of dental caries in the tooth entities of the deciduous dentition. Average caries counts of upper and lower pairs of teeth.

The first fillings were recorded in the 4-year-old group. The increase in the number of fillings, however, is insufficient in relation to the caries increment.

Upon entering school, only 8.6 per cent of the children are free of dental caries; the df count amounts to 4.85. The count consists of 78, 3 per cent caries, 16, 8 per cent root remnants, but only 4.9 per cent fillings. The low count of fillings indicates that no systemic treatment is afforded to children of preschool age. Apparently, they are brought to the dentist by the parents only in the case of an emergency. Undoubtedly, the treatment of preschool children is undertaken by most dentists for the sole purpose of eliminating pain.

4. *The degree of dental caries:* An indicator of the progress of caries and the amount of destruction of the teeth can be seen from the data in Table V. As the number of superficial carious lesions decreases, an increase occurs in the number of medium and deep caries.

TABLE V
PERCENTAGE DISTRIBUTION OF THE SEVERITY OF CARIES IN THE DECIDUOUS TEETH

	NEXT BIRTHDAY						TOTAL
	2	3	4	5	6	7	
Superficial	96.0	89.0	38.5	24.9	16.0	17.9	22.0
Medium	4.0	10.4	52.7	62.5	68.2	62.8	61.0
Deep	-	0.6	8.8	12.6	15.8	19.3	17.0

At 3 years of age, 89 per cent of all lesions are superficial, and deep caries are found in only 0.6 per cent of the decayed teeth. In the 4-year-old children, a sudden deterioration is obvious: 35.8 per cent superficial, 52.7 per cent medium, and 8.80 per cent deep caries are found. Thereafter, a steady decline occurs; at 7 years, superficial caries are found in only 17.9 per cent, medium caries in 62.8 per cent, and deep caries in 19.3 per cent.

It was pointed out in the preceding paragraph that there is no obstacle to inserting fillings in children of 4 years. The deterioration of the deciduous dentition starting at the same age demonstrates the necessity of inserting fillings. *If systemic treatment of the deciduous dentition is not begun in the fourth year, one will be unable to cope with the accumulation of caries progression.*

5. *Caries prevalence in the single units of the deciduous dentition* (Fig. 1): None of the tooth entities is endowed with a resistance to decay.

a. Caries is most prevalent in the second deciduous molar. This tooth contributes 40 per cent to the total caries experience of the deciduous dentition. In decreasing frequency it is followed by the first molars (27.5 per cent), central incisors (17.0 per cent), lateral incisors (10.8 per cent), and cuspids (4.8 per cent). The distribution of caries among the different tooth entities is similar to that experienced in the permanent dentition.

As in the permanent dentition, caries attack those deciduous teeth which erupt first. In children of 2 years of age (at this age eruption of the deciduous molars is in progress), the anterior teeth are more heavily attacked than the posterior teeth. Sixty per cent of the total caries experience is contributed by the anterior teeth, and only 40 per cent by the posterior teeth. In the next

Fig. 2.—Prevalence of caries in the upper and lower deciduous teeth.

year, a heavy attack of the deciduous molars occurs; a 62 per cent caries frequency of these teeth is observed. In children of 4 years of age, by increasing attack rates of the first deciduous molars, this percentage figure increases to 70. Thereafter, no conspicuous change occurs but, nevertheless, an increase of the molar attack rate is observed from 70 to 80 per cent, up to 7 years of age.

b. Caries prevalence in upper and lower deciduous teeth (Fig. 2). Separating caries prevalence figures of the upper and lower deciduous teeth, one observes a heavier decay of the lower molars and of the upper anterior teeth.

In children of 2 and 3 years of age, no caries were observed in lower incisors. Even in the later years, lower incisors contribute somewhat less than 10 per cent to the total caries experience of the incisor region.

Caries in the lower cuspids starts at an early age, and of all lower anterior teeth, cuspids are the most heavily attacked by decay. At 3 years of age, lower cuspids contribute approximately 33 per cent to the total caries experience of the canine area; at 4 years, 13.3 per cent; and in the later years, approximately 20 per cent.

There is no conspicuous difference in the caries attack rate of the lower first and second deciduous molars; in these teeth, the prevalence of caries is of approximately equal severity. Mandibular molars become carious earlier than maxillary molars. At 3 years of age, 75 per cent of the total deciduous molar caries experience is contributed by the lower teeth and only 25 per cent by the upper teeth. With increasing age, there is a trend toward eliminating this difference, but even at 7 years of age, 32 per cent is the attack rate of the lower molars. Similar but less marked differences were stated by Cohen,[5] and Cohen and Green[6] between the caries attack rates of upper and lower deciduous molars. The heavier decay of the lower molars somewhat implies acquired malocclusion. In consequence of early decay and early loss of the lower deciduous molars, the first permanent molars often suffer a mesial shift, resulting in malocclusion.

c. In contrast to the caries of the permanent dentition, there are some features of deciduous decay to which we intend to direct some attention: (1) In the permanent dentition, decay of the anterior teeth starts at a later age than that of the posterior teeth. In the deciduous dentition, caries of the anterior teeth begins at an earlier age. (2) In the permanent dentition, the preponderance of the multicuspidated posterior teeth is maintained constantly throughout the life span. At 3 years of age, a change occurs in the deciduous dentition; the posterior teeth surpass the front teeth in the prevalence of decay. This item deserves consideration from an etiological point of view. (3) Apparently, the deciduous cuspid is not endowed with the same resistance to decay as is its permanent successor. Caries in the deciduous canines is seen as early as 2 years of age: In spite of this early decay, the most resistant deciduous tooth entity is the cuspid. Its attack rate, amounting to 7 per cent in the juvenile years, is maintained at the same level with advancing age.

6. *Circular caries* (Table VI): Circular caries prevails in 7.94 per cent of examinees.

We were interested furthermore in the question, What percentage of the caries in the anterior teeth is of the circular type? At 2 and 3 years, nearly

all incisor caries are of this kind. Approximal caries is observed in the anterior teeth from the fourth year on.

Comparing the data of Table VI with Fig. 2, one becomes aware that, in children, decay starts as circular caries of the upper anterior teeth. The initial caries attacks occur in the fissures as well as on the proximal surfaces of the deciduous molars at approximately the same time.

TABLE VI

PERCENTAGE VALUES OF CIRCULAR CARIES IN THE UPPER ANTERIOR TEETH (RELATED TO THE TOTAL CARIES EXPERIENCE OF THE SAME TOOTH TYPE AT THE SPECIFIED AGE)

	NEXT BIRTHDAY						TOTAL
	2	3	4	5	6	7	
Central incisor	100.0	100.0	42.1	26.3	22.9	3.8	48.8
Lateral incisor	100.0	100.0	68.0	42.2	42.0	5.3	59.5
Cuspid	100.0	100.0	30.7	33.5	21.8	2.6	47.5
Totals	100.0	100.0	48.4	32.4	27.8	4.1	51.9

7. *Four-year-old children* (Fig. 3): Some periods of human life are regarded as of special importance in relation to the dentition; the age of 6 or 24 months, of 6 and 14 years, etc. In studying the development of caries in the deciduous dentition, a further milestone of importance becomes obvious—this is the fourth year of life. What is the special significance of this age? The answer is found in Fig. 3.

With the progress of caries in the deciduous dentition, there is a steady increase of the df count, of the number of medium and deep carious lesions, and of root remnants. At the same time, a decrease is observed as to the number of caries-free individuals and number of superficial carious lesions. Crossing of increasing and decreasing curves occurs in the fourth year of life (between the third and fourth birthdays); children with caries in the deciduous teeth outnumber those who are caries free; more teeth display medium, or deep, than superficial caries. In the fourth year, a relative decrease occurs in the number of circular caries preponderant.

These findings point to the fourth year of life as the critical one for the deciduous dentition. At this particular age the predominant factors that are causing circular caries are stopped, other factors, only partly known, are coming into the scene and causing carious lesions of other types. At this particular age, systemic treatment of the deciduous dentition must be begun. Caries-preventive measures are to be instituted prior to this age in order to obtain any result in this age bracket. The effects of such measures can easily be estimated by postponing the "critical" age or by its eventual disappearance.

According to our present knowledge, there is no difference in the etiology of caries in the deciduous and in the permanent teeth, that is, with the exception of circular caries, which we believe to be etiologically different from all other types. The study of the carious lesions in the deciduous teeth offer better prospects in regard to caries-preventive experiments with human beings because of the shorter life span of the milk-dentition. The total life span of the deciduous dentition amounts to hardly more than 10 years, whereas multiples of a decade are to be considered as life span of the permanent dentition.

B. The Permanent Dentition.—

The study of caries in the deciduous dentition was our primary aim. In regard to the permanent teeth, we should like to emphasize one finding only, i.e. carious lesions start immediately after the eruption of the first permanent teeth.

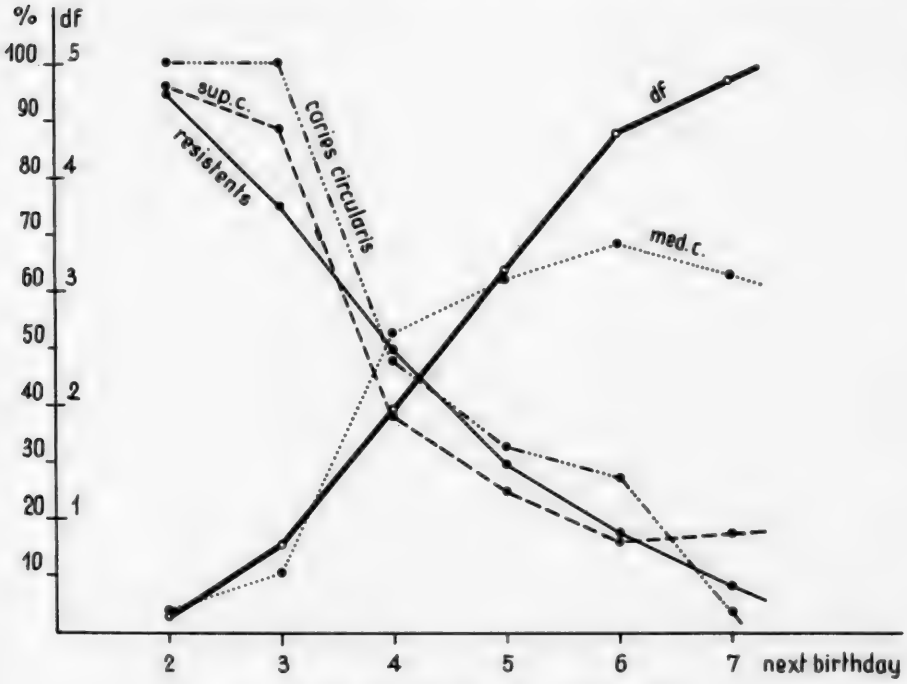

Fig. 3.—The importance of the "critical" year.

DISCUSSION

Literature prior to 1945 indicates a high degree of decay in preschool children.

The frequency of caries prevalence in the United States amounts to 58 per cent, according to McCall[7]; in England in children under 2 years of age to 2.3 per cent, according to Doherty[8]; and to 26 per cent among 3-year-old children in England according to Pitts.[9] Jensen[10] found that in Copenhagen the caries activity of 6-year-olds reached 82 per cent. McCall's material is so selected that no general validity can be attributed to his findings.

Further data are published as to caries prevalence of preschool children: Ast[11] in the United States found that 60.2 per cent of the children from 1 to 5 years displayed caries activity; Jessen in Strassbourg,[12] 84 per cent of the 3- to 6-year-old group; Doherty,[8] in Birmingham, 52.3 per cent of the 4- to 5-year-old group.

TABLE VII
PREVALENCE OF DENTAL CARIES IN PRESCHOOL AGE GROUP*

AUTHORS	SITE OF EXAMINATION	YEAR OF PUBLICATION	NUMBER OF EXAMINEES	df COUNT OF CHILDREN (YEARS OF AGE†)						
				1	2	3	4	5	6	7
Finn[38]	Newburg, N. Y. Kingston, N. Y.	1947	938			0.31	1.13	2.58	4.64	
Chilton and Greenwald[39]	North Arlington, N. J.	1947	70						3.25 (12)	3.75 (58)
McLaren[20]	Canada	1948							3.40	4.50
Wisan and Chilton[40]	New Jersey	1948	8,842					3.10 (60)	3.62 (3,418)	4.24 (5,364)
Dean, Arnold, Jay, Knutson[20]	Grand Rapids, Mich.	1950						2.70	3.30	4.60
Dean, Arnold, Jay Knutson[20]	Muskegon, Mich.	1950						4.40	5.60	6.00
Witter[20]	Tigard, Ore.	‡								5.26
Fulton[10]	Illinois	1952	3,009	0.00 (163)	0.02 (313)	0.22 (285)	1.06 (277)	2.82 (433)	4.07 (1,538)	
Fulton[10]	Cleveland, Ohio	1952							5.38	6.19
Witter[20]	Bend, Ore.	§								5.03
Hadjimarkos[20]	Corvallis, Ore.	1953							4.48	5.67
Cohen and Green[6]	Brookline, Mass.	1954	443					2.89 (347)	4.06 (88)	
Savara and Suher[20]	Portland, Ore.	1954	650		0.67 (18)	0.83 (65)	2.72 (123)	4.05 (134)	4.76 (184)	5.13 (126)
Wisan, Lavell, Colwell[31]	Philadelphia, Pa.	1957	2,650			0.60 (201)	2.20 (308)	3.60 (636)	3.50 (1,460)	

Note: The numbers in parentheses under "df Count of Children" are of the number of examinees of the specified age group if known to us.

*Nonuniform criteria have been studied and some of the differences are simply due to differences in the various definitions of a carious lesion.

†Age next birthday: In this way, comparison of Fulton's and our results is possible.

‡Examinations were carried out in 1952.

§Examinations were carried out in 1953.

The number of children with caries experience varies at 2 years between 13.9 and 34.7 per cent,[13] at 3 years between 24.3 and 73.5 per cent,[11, 14-21] at 4 years between 38 and 85 per cent,[11, 18] at 5 years between 56 and 95 per cent,[11, 13, 18, 21, 22] at 6 years between 60.9 and 97.6 per cent.[14-17, 19, 23]

The frequency of carious teeth was found to be 5 per cent at one year[24]; between 13.9 and 37.3 per cent at 2 years[13, 24]; between 9.3 and 29.7 per cent at 3 years[13-15]; 40.1 per cent at 4 years[13]; between 20 and 49.2 per cent at 5 years[13, 25]; and between 16.1 and 58.2 per cent at 6 years.[13-15]

Hamill and Sausser,[26] in 1944, found that the prevalency of carious lesions in white and Negro children at one year of age was 0.95 per cent; at 2 years 1.98 per cent; at 3, 3.7 per cent; at 4, 4.30 per cent; at 5, 5.70 per cent. Sebelius[27] concluded that Negro children in the age group from 3 to 5 have better teeth than white children (def 2.22 and 4.60, respectively). However, the number of examinees was rather low.

In Table VII we compiled the more recent data of investigations upon the prevalence of dental caries in the deciduous teeth relying upon available literature. In these investigations methods similar to ours were used.

At 6 months of age, no carious lesions were observed by Fulton[4] in 163 examinees. By the first birthday, however, caries were prevalent. In our opinion, the first caries attack occurs prior to the first birthday. This is obvious from Jones, Larsen, and Pritchard's papers[28, 29] where it is shown that caries is prevalent in one third of children between 6 and 12 months. The amount of caries displays some variation in the different geographical areas and populations, but seems to be uniformly rather grave. A rapid increase between the third and fourth birthdays is obvious in all reports: Fulton found an increase from 0.22 to 1.06; Savara and Suher[20] from 0.83 to 2.72; Wisan and associates[31] (1957) from 0.60 to 2.20 of the df count. *The critical year is present everywhere!* It seems probable that it depends on caries activity. In populations over the world with similar caries activity, the fourth year of life seems to be the critical one uniformly.

The prevalence of caries in the anterior deciduous teeth assumedly points to the deleterious effects of certain oral habits. The total devastation of the front teeth to root remnants by circular caries in children of 3 years of age is caused by the use of the titty-bag[32] and similar sugar-filled comforters. By abolishing the use of such comforters, we could probably gain 2 years for the deciduous dentition and decrease considerably the amount of decay in children below 3 years of age. In our own material, by subtracting circular caries in the 2-year-old, a df count of 0.06 remains; in 3-year-olds, one of 0.40. This points to be beginning of heavier decay in the deciduous dentition after passing of the second birthday, and the cessation of the eruption of the deciduous dentition. In this way, the fourth year of life, the critical year, would gain additional significance.

Prophylaxis, however, must attack all types of caries and not be restricted to the circular caries. As regards prophylaxis in the deciduous dentition, stress must be given to the importance of proper nourishment. We should like to recommend certain publications which elaborate on this particular topic.[33-37]

At what age can dental treatments be started in children? Our results emphasize that systemic treatment has to start in children having passed their third birthday. However, in our opinion, more success may be expected in the fight against caries of the deciduous dentition from prophylactic means than from therapeutic measures.

SUMMARY

The prevalence of caries in the deciduous teeth of 2,699 children of both sexes between 2 and 7 years of age, living in Szeged, Hungary, was investigated. No difference was found in the prevalence of caries between the sexes. The df count is 0.15 per cent at 2 years, and 4.85 per cent at 4 years. In the deciduous dentition, caries starts prior to the first birthday, by circular decay of the anterior teeth. In regard to the caries attack rate, great importance is to be attributed to the fourth year of life, which we have labeled "the critical year." Stress must be laid upon prophylaxis, in combating caries of the deciduous dentition, rather than on therapeutic measures.

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COMPARATIVE EFFECTS OF FLUORIDES ON MICE FEMURS

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INTRODUCTION

ALTERATIONS in the bony skeletal structure may be reflected histologically in the cell-dividing zone, the proliferating zone, and the hypertrophic cell zone of the epiphyseal plates or growth centers of the long bones.[1-4] Particular conditions inducing such changes may result from modifications in diets, vitamin intakes, or hormones.[1, 5-12] In addition, there has been an increasingly widespread interest concerning the effect of fluorine on the mineralized structures of man, cattle, and experimental animals.[13-15] Despite this, the in vivo mechanisms of fluorine or of fluoride compounds are not clearly understood.

Concepts that different fluoride compounds can exert different biologic effects on bone have not been adequately explored, although numerous investigations have dealt with changes in tissues following fluoride administration. Usually dosages were high, animals used were few, and comparisons were not made on a biologic basis between different fluoride compounds. Observations have not been reported with regard to histologic changes associated with growth centers in bone where different fluoride compounds have been used. It is of interest to compare the effects of several fluoride compounds. For such a study, mice femurs are ideally suited. The fluoride compounds used in the present report were NaF, CaF_2, Na_2SiF_6, and KF.

This communication constitutes a report on the effect of the above-mentioned fluoride compounds on mice femurs.

METHODS

Young adult dba Rockland mice, of similar weights and ages, equally divided and separately maintained as to sex, were used for these experiments.

The different conditions under which (see Table I) these experiments were carried out were as follows:

1. Normal mice; serving as controls and on tap water.

2. Mice on distilled water; given distilled water ad libitum via their drinking water for 45 days.

3. Mice on fluorides; given the following fluoride compounds—NaF, CaF_2, Na_2SiF_6, and KF ad libitum via their drinking water for 45 days; concentra-

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tions of solutions were 1:100,000, by weight with distilled water, or 10 ppm of the whole salt.

In each of the fluoride categories, as well as in the distilled water and control groups, 5 males and 5 females were used. In this manner, 20 complete series were run. In addition, reserves were set up for each category to replace lost or moribund animals and maintain total group numbers for the duration of the experiment.

Animal weights were recorded three times weekly. Fluid consumption was measured directly and recorded from graduated drinking bottles three times weekly. At such times, bottles were cleaned and refilled with fresh solutions. Animals were fed ad libitum on standard Purina Laboratory Chow. Tap water determination for fluorides was 0.0017 ppm.

Animals were sacrificed after 42 to 45 days. The groups represented in Table I were sacrificed at 45 days. Autopsies were performed immediately after sacrificing. Femurs were freed of adhering flesh and placed in Zenker's solution for 12 hours, removed, and washed in tap water for 24 hours, and then placed in 80 per cent alcohol for 24 hours before decalcification. Sectioning was usually at ± 7 μ. Staining was with hematoxylin and eosin.

TABLE I

GROUP	SEX & NO.	INITIAL WEIGHT OF GROUP (GM.)	FINAL WEIGHT OF GROUP (GM.)	DIFFERENCE (GM.)	FLUID CONSUMPTION 45 DAYS (C.C.)	FLUID CONSUMPTION AVERAGE/DAY/MOUSE (C.C.)
Tap	5 M & 5 F	209.5	221.9	+12.4	1,826.5	4.13
Distilled	5 M & 5 F	209.2	222.4	+13.2	1.809.0	4.02
NaF	5 M & 5 F	206.4	205.0	− 1.4	1,721.5	3.47
CaF_2	5 M & 5 F	209.3	265.4	+56.1	2,295.0	5.1 †
Na_2SiF_6	5 M & 5 F	204.0	211.0	+ 7.0	1,496.25	3.32
KF	5 M & 5 F	209.1	239.0	+28.9*	2,380.5	5.55‡

*With KF, 4 died in this group and had to be replaced: also 4 reserves died.

†There was a marked increase in weight of CaF_2 mice over the experimental periods. This was also manifested by a marked increase in the thickness of the lateral borders of the shaft of the femurs.

‡Initial intake for first 7 days averaged almost 7 c.c. daily per mouse, after which some became moribund and intake dropped sharply.

OBSERVATIONS

Femurs from mice receiving the fluoride compounds and distilled water showed alterations in terminal plates, epiphyseal cartilage, trabeculae, and blood vessels. These observations are summarized in Table II.

Terminal Plates.—Lamellae in terminal plates along the proximal aspect of the epiphyseal cartilage from animals on distilled water were broader, closer together, and considerably more irregular than in the tap water control animals. Thicker terminal plates were observed in animals on distilled water. This can be determined by comparing Figs. 1 and 2. Terminal plates were thinnest in NaF treated mice where the lamellae were finer and spacing varied considerably. It also appeared that the maturity of this osteoid tissue varied considerably in such instances. With CaF_2 treated animals, lamellae were usually more

TABLE II
SUMMARY OF RESULTS

GROUP	TERMINAL PLATES	TRABECULAE OF METAPHYSES	EPIPHYSEAL CARTILAGE	BLOOD VESSELS
Tap water	Sharp demarcation with cartilage	Cartilage cores; no clear margins	Cartilage cells maturing and changing into osteoid; generative area active	Along distal border of plate; none within the cartilage
Distilled water	Broad close irregular lamellae; thicker and irregular in places	Thinner with some microfractures; formation ragged and calcification poor; small cartilage cores	Numerous areas without cartilage cells, more immature	Similar to mice given tap water except that some vessels lay within the cartilage along the distal border
NaF	Finer lamellae with irregular spacing and staining; thinner and poor calcification	Ragged, larger, less numerous; poor calcification	Lining up of cartilage cells increased; larger cells than in the controls	Less numerous; broken endothelium seen in places
CaF_2	Lamellae more poorly defined and uneven but broader than with the other fluorides; thicker	Fewer, heavier and broader lamellae; large clear areas; cartilage cores deep in metaphyses	Cartilage cells attained a large size; numerous clear acellular areas; fewer mature cells	More numerous and larger, well-filled with RBC; some within cartilage
Na_2SiF_6	Lamellae thin and nonexistent in places; spacing and staining variable; thinner	Variable staining, very dark, ragged and irregular; some microfractures; numerous clear spaces	Thinner; active layer shallow; cells border directly on marrow	Fewer and smaller with less RBC; some broken walls of endothelium
KF	Lamellae sharp with variable width, deep staining, sharp delineation with cartilage	Fewer in number; lighter staining; no deep cartilage cores	Disorderly cell formation; small cells; thin	Less numerous and some lay in distal border of cartilage

poorly defined and uneven but broader than in instances where treatment was with other fluorides. With KF treated animals, lamellae were more sharply defined, more variable in width, and staining was deeply colored by hematoxylin along the epiphyseal cartilage margin. The thickest terminal plates were found where CaF_2 had been given. This can be observed by comparing Fig. 4 with Figs. 1, 2, 3, 5, and 6. The thinnest terminal plates were found in NaF (Fig. 3) and Na_2SiF_6 (Fig. 5) treated femurs. In places with NaF and Na_2SiF_6, the terminal plates were often nonexistent.

Trabeculae.—Animals on distilled water had thinner trabeculae than were found in the tap water controls. This can be observed by comparing the trabeculae in Figs. 1 and 2. Microfractures were evident in the narrower places of the more unevenly formed trabeculae in the animals given distilled water, and cartilage cores were much smaller or nonexistent deep within the trabeculae. Where NaF had been given, as shown in Fig. 3, the trabeculae were ragged along the borders and in larger formation at some distance from the epiphyseal

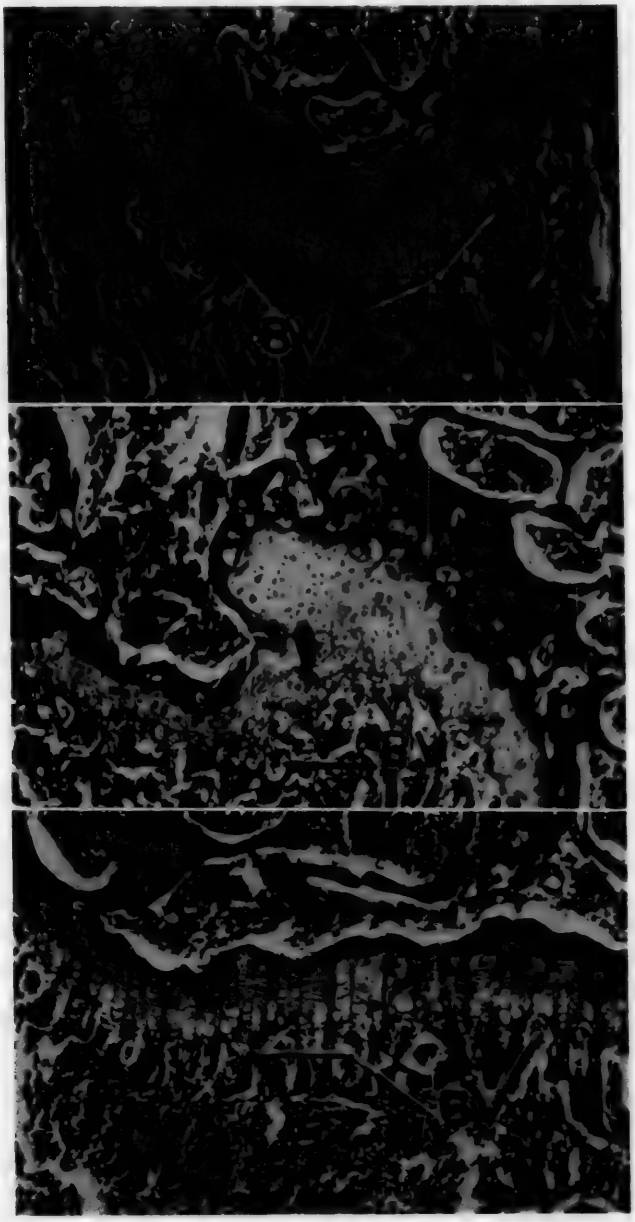


Fig. 1.

Fig. 2.

Fig. 3.

Terminal plates to the *top* and trabeculae to the *bottom* of all figures.

Fig. 1.—Normal tap water control—growth center of femur of male mouse. (Orig. mag. × 310.)

Fig. 2.—Distilled water—growth center of femur of male mouse. (Orig. mag. × 310.)

Fig. 3.—NaF treated—growth center of femur of female mouse. (Orig. mag. × 310.)

Fig. 4.

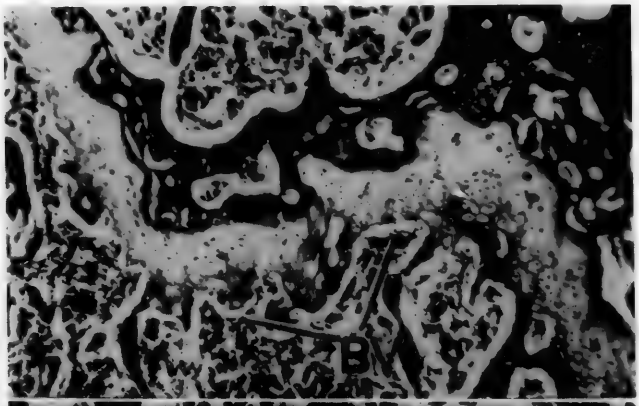


Fig. 5.

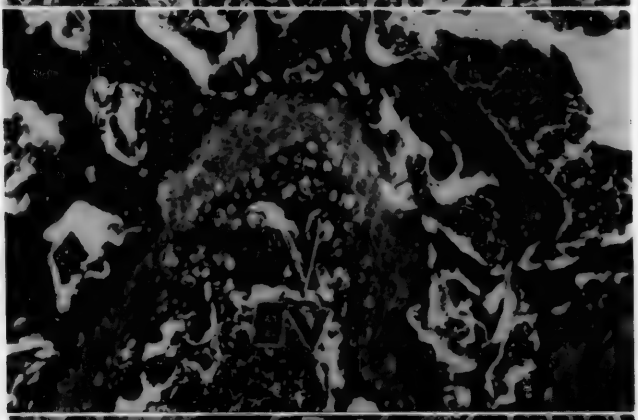


Fig. 6.

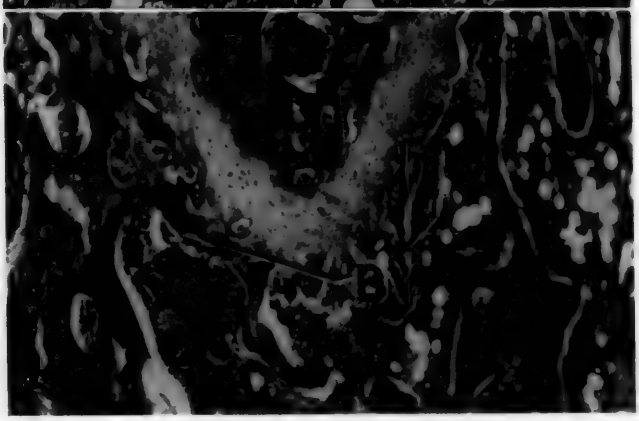


Fig. 4.—CaF_2 treated—growth center of femur of male mouse. (Orig. mag. × 310.)
Fig. 5.—Na_2SiF_6 treated—growth center of femur of female mouse. (Orig. mag. × 310.)
Fig. 6.—KF treated—growth center of femur of female mouse. (Orig. mag. × 310.)

cartilage. In femurs from CaF_2 treated mice, trabeculae were fewer and thicker, with remnants of calcified cartilage or cartilage cores well down into the metaphyses. This is shown in Fig. 4. At times, microfractures were observed in femurs of CaF_2 treated mice. There were usually more microfractures associated with trabeculae in femurs of Na_2SiF_6 treated mice than with the femurs from those treated with distilled water. Where microfractures were evident, trabeculae were surrounded with young connective tissue. With Na_2SiF_6 (Fig. 5), staining of trabeculae was deeper but numerous areas of associated calcified cartilage gave them an uneven appearance. With KF (Fig. 6), trabeculae were fewer in number and took a lighter stain, and cartilage cores were not found deep in the metaphyses. In general, with the exception of CaF_2, where fluorides had been given, calcified cartilage was not found deep in the metaphyses. When present, however, it was located in the trabeculae near the epiphyseal border.

Epiphyseal Cartilage.—Several significant differences were observed within the epiphyseal discs under the several different categories of these experiments. Variations in the thickness of the epiphyseal discs were noted. It is readily apparent that Figs. 3 through 6, representing the fluoride treated mice, show thinner epiphyseal discs. By comparing Figs. 7 through 12, it is likewise evident that, where treatments were given, cellularity was decreased. Furthermore, except for NaF treated animals, chondrocytes were considerably less regular in arrangement. In all instances of fluoride treated mice, there was a marked retardation of aging, for chondrocytes appeared to partially degenerate but were apparently blocked during the latter stages of their life cycles. This is evident in Figs. 8 through 12 when they are compared with Fig. 7 which is of the tap water control. With CaF_2, the consistency of the cartilage plate was altered and it appeared fibrous. In addition, as seen in Figs. 4 and 10, mature osteoid formations were found well within the epiphyseal cartilage plates. This was similarly observed, but less frequently, where distilled water had been administered.

Blood Vessels in Relation to the Distal Border of the Cartilagenous Plate.—Blood vessels are designated in the figures by the drawn lines from the letters *BV*. In the tap water controls, small blood vessels were present in a continuous and a contiguous relationship along the distal border of the cartilagenous plates, between the trabeculae. Blood vessels were not found, as shown in Fig. 1, within the epiphyseal cartilagenous plates. Vessels were filled but not engorged with red blood cells. A similar arrangement was observed where distilled water had been given, but a number of the smaller vessels appeared within the distal cartilagenous borders, as seen in Fig. 8. This is also seen in Fig. 2.

Where NaF had been given, blood vessels were comparatively less numerous. Some appeared to have broken endothelial walls and, as a result, red blood cells were observed in surrounding areas. Blood vessels were most numerous where CaF_2 had been administered (Figs. 4 and 10). In such instances, the lumens were generally large and well filled with erythrocytes (see Fig. 10)

Fig. 7.

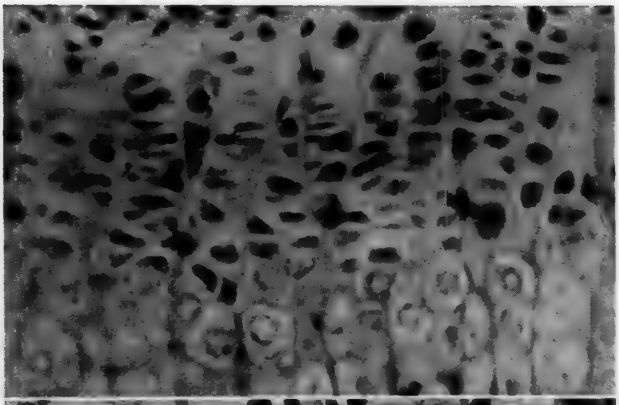
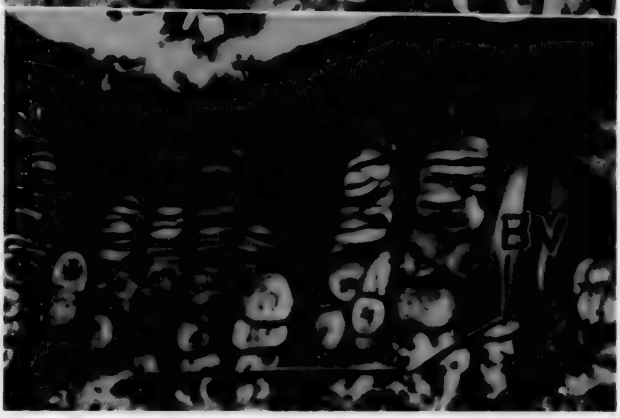


Fig. 8.

Fig. 9.



Figs. 7-12 are taken at the junction of the metaphyseal border.
Fig. 7.—Higher magnification of Fig. 1 to show cartilage plate. (Orig. mag. × 1,430.)
Fig. 8.—Higher magnification of Fig. 2 to show cartilage plate. (Orig. mag. × 1,430.)
Fig. 9.—Higher magnification of Fig. 3 to show cartilage plate. (Orig. mag. × 1,430.)

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 10.—Higher magnification of Fig. 4 to show cartilage plate. (Orig. mag. × 1,500.)
Fig. 11.—Higher magnification of Fig. 5 to show cartilage plate. (Orig. mag. × 1,430.)
Fig. 12.—Higher magnification of Fig. 6 to show cartilage plate. *BV*—blood vessel. (Orig. mag. × 1,500.)

and some vessels lay well within the epiphyseal cartilage discs. Where this occurred, the cartilage appeared like osteoid tissue and stained deeper, more with hematoxylin than eosin.

With Na_2SiF_6 (Figs. 5 and 11), blood vessels were fewer and smaller. As with NaF (Figs. 3 and 9), some vessels appeared disrupted and occasionally were found within the border of the epiphyseal cartilage. Where KF had been administered, vessels were also less numerous and, as with NaF, they were likewise within the distal borders of the epiphyseal cartilage plates (Figs. 6 and 12). In addition, with KF there were large vacuolar areas near these vessels.

Other Observations.—The greatest increase in weight (usually marked) occurred with CaF_2. When KF was given, consumption was high, initially averaging more than 6.3 c.c. per mouse for the first 7 days. Soon this dropped and, by 8 to 10 days, dead or moribund mice that had to be replaced were found in the cages. It was difficult to keep all animals alive with the 1:100,000 KF for the 45 days in this part of the experiments.

DISCUSSION

The purpose of this study has been to make a comparison of the effect of administration of several fluoride compounds on the epiphyses of mice femurs. Alterations taking place within bony skeletons are manifested by changes in this region.[1-4] Histologic data obtained in this study suggest that the fine metabolic balance between the continuous formation of cartilage and its destruction at the metaphyseal border can be upset by these fluoride compounds. In addition, with the possible exception of NaF and Na_2SiF_6, different effects were exhibited in this area.

With mice on distilled water, it was evident that the absence of salts or minerals in the drinking water likewise had a definite effect which was reflected within the epiphyseal cartilage plate. Variations in thickness, decreased number of chondrocytes in the generative layer, acellular areas, small osteoid formations within the epiphyseal plate, and encroachment of small blood vessels were some of the manifestations of a metabolic upset in these instances. It is well known that the presence of blood vessels enhances the change of cartilage to bone.[1-4, 25] This may have been the reason that small osteoid formations were found well within the cartilage plate where distilled water had been given.

McClure compared the physiologic effects of NaF and Na_2SiF_6, expressing the opinion that any differences in their effects would depend upon absorbability when large quantities were ingested.[9] He felt, however, that differences related to absorbability would be markedly minimized when very dilute solutions were administered. In addition, he could ascribe no noticeable differences to the route of administration, whether it was by food or by drinking water.[16] The anticipations of McClure,[9] with regard to minimizing any differences in the physiologic effects of very dilute solutions of NaF and Na_2SiF_6, are partly supported in the present study by virtue of several common histologic findings. Although microconcentrations of several different compounds were used in another recent study, emphasis was on recovery, which was considered good, rather than on biologic effects.[12]

It will be difficult to explain the role of inorganic ion exchange in the metabolism of living bone or cartilage until further explanations are forthcoming on a physicochemical basis. It is generally accepted that the body strives to maintain a constant level of Ca^{++} and other inorganic ions within its tissues.[3] Differences in the physiologic effects of these fluoride salts on the epiphyseal plates of mice femurs may be related to an upset in the intra- and extracellular ratio of cations and anions. Norman and associates[17] determined the ratio of Ca^{++}, Na^{+}, and K^{+} in bone, and expressed it in milligrams as follows: 200 ($Ca_3[PO_4]2$); 4 (NaCl) and 1 (KCl). In another report, the differences in biologic effects on enamel protein between fluoride salts with Na^{+} and Ca^{++} cations was explained on the basis that Ca^{++} and F^{-} are both antagonistic to Mg^{++}, while Na^{+} is not.[18] It has likewise been established that Na^{+} ions inhibit the activity of K^{+} by replacing it on the enzyme protein in a stepwise conversion to high energy phosphate.[19] Presumably where KF is used, as in the present study, the ratio of Mg^{++} to ATP is increased when the concentration of K^{+} is increased. However, this relationship could not be entirely accomplished as long as there are free F^{-} ions which are antagonistic to Mg^{++}. Changes in the epiphyseal plates of the mice femurs may thus result from alterations in the extracellular and intracellular ratio of cations,[17] competition between cations or anions that are antagonistic to Mg^{++},[12, 18] inhibition or activation of high energy phosphate conversion by competition or antagonism of cations for places on the enzyme protein.[19]

The decrease in thickness of the terminal plates, in the animals treated with NaF, Na_2SiF_6, and KF, is believed to have resulted from a retardation in the osteoblastic activities of bone-forming cells in this layer. Thus, the conditions in the present instances (where epiphyseal plates are thinned with administration of NaF, Na_2SiF_6, or KF) were presumably similar to those defects in cattle described by Rand and Schmidt.[10] Among their findings, they observed lameness and stiffness when cattle received 2 mg. per kilogram of body weight of soluble fluoride daily. In contrast, however, where CaF_2 had been given, thickness of terminal plates increased, presumably from enchancement of osteoblastic activity. This was further emphasized by the greater weight of animals so treated.

In the fluoride (except between NaF and Na_2SiF_6) treated animals, a marked difference in maturation of chondrocytes within the epiphyseal cartilage gives further support to a dissimilarity of effects of these compounds. Changes in epiphyseal cartilage become evident under several conditions, among which are excess vitamin A,[7] avitaminosis D,[4] restricted diets,[1] and hormonal influences.[4, 19, 21, 22] It has been suggested that maintenance of a high level of chondroitin sulfate is a device by which cartilage may be protected against calcification.[3, 7, 23, 24] Since it is known that in vitro calcification of cartilage can be blocked, at least in part, by enzyme inhibitors,[3] the present findings indicate that the F^{-} ion, presumably acting as an inhibitor, resulted in an alteration of the cycle of maturation and of degeneration of chondrocytes. Apparently this was also paralleled by the thickness of the epiphyseal plates. Both Belanger and Comar and their associates noted a distortion in the linear

pattern of chondrocytes where 1,000 ppm of fluorides were given to pigs.[2, 3] In the present experiments, where NaF and Na_2SiF_6 had been given, the linear pattern was more regular than that seen in the other instances of the present experiments. This regularity in cell alignment was reminiscent of protein patterns obtained in Zone A of the enamel of the mouse incisor when these same substances were administered.[18] It is also likely that higher concentrations of NaF, as used by them,[5, 6] did not enhance an orderly arrangement of chondrocytes.

The epiphyseal cartilage plates were wider with CaF_2, and the linear pattern of chondrocytes was not orderly in formation. In such instances, their arrangement was more like the formations seen in the control animals, or those given distilled water. Areas devoid of cartilage cells, which assumed a fibrillar appearance with CaF_2, could have been additional manifestations of structural alterations that this substance induced within the cartilage plates. It is interesting to note that, despite these alterations, there was at no time the extreme widening of epiphyseal cartilage discs described by Comar and associates.[6]

Retardation of the maturation of the epiphyseal cartilage was reflected in the trabeculae and also in their relationship to the metaphyseal border. It was pointed out by Ring[25] that mesenchymal cells invading the epiphyses were directly related to the increasing metabolic demands of an increasingly large mass of cartilage. In addition, Ring[25] found a direct relationship between the vascularity and the cartilage canals and the hypertrophy or degeneration that precedes ossification. This has likewise been pointed out by LaCroix.[2] Thus, alterations in vascularity along the metaphyseal border have a subsequent effect on the trabeculae. Therefore, wherever vessels are numerous, patent, and well filled with red blood cells, heavier trabeculae formations can be expected to be found. This was true for CaF_2. The important point here is that, with CaF_2, the formations of trabeculae were heaviest in these experiments. Where vascularity was less prominent, as with NaF and Na_2SiF_6, trabeculae were fewer and ragged in appearance, and osteoblastic activity was considerably depressed. With KF, there were similar decreases in the formation of trabeculae, which was presumably due also to a suppression of osteoblastic activity, but the picture differed from that found with either NaF or Na_2SiF_6, for there were more marked changes about the trabeculae. Comparatively, the trabeculae in such instances (KF) had the greatest changes. In addition, the numerous vacuolated and empty spaces about the trabeculae must have resulted either directly or indirectly from increases in K^+ ions.

NaF and Na_2SiF_6 appeared to exhibit the greatest depression on the blood vessels along the metaphyseal border. It was pointed out previously that, along the epiphyseal border, cartilage cells did not degenerate where blood vessels lay within the cartilage.[3] Thus, on the basis of the position of the vessels in relation to cartilage plates, this area was widest when some vessels were found well within the plates of epiphyseal cartilage, as in instances where CaF_2 had been given. With KF, some vessels were found occasionally within the

distal border of the cartilage, but with this fluoride salt, the picture was entirely different, being very dissimilar to that observed in any of the other instances. No remarkable differences were observed concerning the blood vessels between the tap water controls and those animals receiving distilled water.

SUMMARY AND CONCLUSIONS

Groups of mice were given the fluoride compounds—NaF, Na_2SiF_6, CaF_2, and KF—in 1:100,000 dilutions ad libitum via their drinking water for 45 days. In addition, other mice received distilled water. The femurs of animals so treated were compared histologically with each other and with those from the tap water control animals. It was noted that there was a similarity of some, but not all, of the results obtained with NaF and Na_2SiF_6. Also noted, with the exception of NaF and Na_2SiF_6, was a dissimilarity of results obtained with the other fluoride compounds.

Alterations taking place are described with reference to the components of the growth center; the epiphyseal plates, the cartilagenous plates, the trabeculae, and the blood vessels. It was observed that the cartilagenous plates, trabeculae, and terminal plates were heaviest where CaF_2 had been administered. Terminal plates were thinner, cartilage plates were also thinner, but had numbers of cells lined up in columns, and trabeculae were ragged where NaF and Na_2SiF_6 was given. Where KF had been given, there was a very sharp border between the terminal plates and cartilage, trabeculae were fewer in number, and the epiphyseal cartilage was thin with small, disorderly, atropic cell formations. Where distilled water had been given, cartilage was irregular and had variations in maturity, trabeculae were thinner, but terminal plates were thicker and more diverse than with tap water controls. Blood vessels were markedly increased with CaF_2, and they were less numerous than with NaF, Na_2SiF_6, and KF.

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THE RELATIVE LOCAL ANESTHETIC POTENCIES OF PROCAINE, ORACAINE, KINCAINE, AND XYLOCAINE

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INTRODUCTION

THE relative profundity, duration, and rate of onset of dental local anesthetics are usually established by comparative clinical studies. A disadvantage of this procedure is the large number of patients and, necessarily, the length of time required to produce information which is not unduly influenced by injection accuracy, dental problem, patient psychology, and possible prejudicial attitudes by observers.

Laboratory methods of studying local anesthetics quickly provide objective data, amenable to mathematical analysis. Generally, however, anesthetic information is indirect because it is derived from nondental situations.

Worley[1] has extended a method suggested by Everett[2] which permits comparison of local anesthetics in dental situations on dogs. Similar methodology has been employed by Bjorn[3] and Adilman[4] in studies on human beings.

The study reported here was performed to compare the profundity, rate of onset, and duration of four local anesthetic preparations. It also afforded an opportunity to examine the methods in a practical situation.

METHODS

Preparations compared on dogs were: 2 per cent procaine hydrochloride plus 1:50,000 epinephrine; 2 per cent Oracaine HCl plus 1:50,000 epinephrine; 2 per cent Xylocaine HCl plus 1:100,000 epinephrine; 2 per cent Kincaine HCl plus 1:50,000 epinephrine; and 2 per cent Kincaine HCl plus 1:100,000 epinephrine. All but the first named solution (procaine) were also assayed on human beings.

Canine subjects were 5 healthy dogs, each with a shallow amalgam "filling" in a canine tooth. For assay, an animal was lightly anesthetized by intravenous sodium pentobarbital. When so prepared, rubber dam was placed over the tooth to isolate the electrode from contact with any part of the animal's body other than the amalgam to which it was clipped. An indifferent electrode was secured to the skin of a shoulder after it had been shaved and coated with electrode jelly.

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Human subjects were eight volunteers who had a metal, Class I restoration in a vital tooth. Current was applied through an electrode which the subject held in place after the filling had been carefully dried. Cotton rolls were placed to ensure dryness during the brief period of threshold determination. The subject placed one hand in a beaker of saline solution with the indifferent electrode from the stimulator.

Stimuli to dogs were generated by a Harvard inductorium with a 50,000 ohm linear taper potentiometer across the secondary output. The stimuli were applied as brief bursts of tetanizing current before injection, 1, 2, 4, 6, 8, and 10 minutes after injection and every 5 minutes thereafter until the current required to elicit a reflex twitch of the angle of the mandible was again at the pre-injection level, or until 3 hours had elapsed.

Stimuli to human subjects were electronically produced single square waves of direct current, 1 millisecond in duration, occurring every half second.

Injections to all canine subjects were 0.5 c.c. in volume into the mental foramina (after aspiration) in the arch of the stimulated tooth.

Injections into human subjects were also 0.5 c.c. in volume, but were administered as infiltrations (after aspiration) approximating the apices of the stimulated teeth.

Experimental design was carefully planned to eliminate the influence of bias from the operator in both studies and from the subjects in the human series. All injections were coded to enforce this control. To distribute equally to each tested preparation the influence of tolerance, training, scarring, "latin square" treatment sequences were followed in both studies. For those unfamiliar with this procedure, it merely requires that each treatment be first, second, third, and so forth, an equal number of times. In the dog study, each animal started with a different preparation. In the human study, each treatment was a first for 2 subjects, a second for 2 subjects, and so on. In the latter series, the order or sequence of preparations was also varied. In no instance, canine or human, was an experiment performed sooner than 48 hours after the last one, and sometimes the interval between trials was as long as a week.

RESULTS—ANIMAL

The average increments of thresholds over the premedication thresholds were calculated for each animal during each experiment. Recorded in Table I are the average increments of the 5 dogs after each of the 5 treatments at the several intervals after mental injection.

To illustrate the degree of uniformity obtained in this study, the average increment of each animal during the first 30 post-injection minutes was calculated for each of the 5 treatments. These values are presented in Table II. This particular period of time was chosen because it is the usual period of anesthesia desired clinically, as well as the interval during which maximal profundities were obtained and during which no single incident of anesthetic deterioration had begun.

TABLE I

AVERAGE THRESHOLD INCREMENTS (IN VOLTS) OF 5 DOGS OVER PREMEDICATION THRESHOLDS

MINUTES POST-INJECTION	PROCAINE	ORACAINE	XYLOCAINE	KINCAINE 1:50,000	KINCAINE 1:100,000
1	14.2	18.6	19.3	18.7	20.3
2	16.5	20.6	20.9	19.9	21.6
4	19.1	22.6	22.8	21.3	22.7
6	19.6	23.5	23.7	21.9	23.6
8	20.1	24.5	24.8	22.1	24.4
10	20.5	24.6	25.6	22.5	24.7
15	21.5	25.0	26.6	22.4	25.0
20	21.3	24.3	25.4	22.4	24.4
25	19.3	24.6	24.6	22.4	23.5
30	17.4	24.3	23.9	22.1	23.1
35	16.1	23.1	23.5	22.2	22.4
40	14.6	21.7	23.3	21.9	23.3
45	13.1	19.4	22.8	21.8	22.3
50	10.0	13.9	22.4	21.8	20.2
55	6.0	10.2	22.1	21.9	18.0
60	5.4	9.4	21.8	21.6	17.9
65	4.5	9.0	21.8	21.2	17.4
70	2.8	10.4	21.2	21.7	16.2
75	2.0	8.6	20.8	19.3	14.0
80	1.4	8.0	20.4	18.3	13.3
85	1.0	6.9	19.6	17.8	11.5
90	0.7	5.8	18.4	16.6	11.0
95	0.6	5.3	17.0	16.0	10.4
100		5.0	14.3	14.7	10.0
105		4.4	8.6	13.3	9.7
110		2.5	6.5	11.6	9.3
115		1.3	5.4	9.5	9.2
120		1.0	5.0	7.3	9.2
125		0.8	4.7	6.4	8.9
130		0.7	3.1	5.8	8.7
135		0.7	2.4	5.4	8.1
140			1.6	5.1	7.6
145			1.2	5.0	7.3
150			0.8	4.5	7.1
155			0.6	4.1	6.9
160			0.6	3.7	6.5
165				3.2	6.3
170				2.6	
175					

TABLE II

AVERAGE THRESHOLD INCREMENT OF EACH DOG FOR EACH TREATMENT DURING FIRST 30 MINUTES AFTER INJECTION

DOG	PROCAINE	ORACAINE	XYLOCAINE	KINCAINE 1:50,000	KINCAINE 1:100,000
1	21.2	27.3	22.2	23.3	15.3
2	20.8	24.6	24.4	21.4	22.3
3	10.3	26.3	24.1	18.8	23.7
4	23.9	27.6	27.7	28.6	29.0
5	18.5	10.4	20.4	15.9	26.4

In order to determine the differences attributable to the drug effects, statistical analyses were performed. An analysis of variance was performed on the data for each interval up to 90 minutes after injection. These analyses are summarized in Table III. The profundity of procaine-induced anesthesia,

in this study, was significantly inferior to the effect of the other four preparations in the first and second minutes after injection; that is, the onset was definitely slower. Statistically significant differences between the effects of the five compounds did not appear again until 40 minutes after injection when the procaine anesthetic was rapidly diminishing. Procaine and Oracaine threshold increments did not differ significantly from each other from 45 to 90 minutes after injection; neither were there significant differences between increments produced by the other three compounds during the same interval. However, the procaine- and Oracaine-induced increments were significantly inferior to the Kincaine- and Xylocaine-produced increments from 45 to 90 minutes after injection.

TABLE III

SUMMARY OF ANALYSES OF VARIANCE OF DOG DATA

POST-INJECTION TIME	MEAN SQUARE OF VARIANCE			TREATMENT VARIANCE IN DETAIL*		TREATMENT VARIANCE IN DETAIL*		
	DOGS DF = 4	TREAT. DF = 4	ERROR DF = 16	BCDE DF = 3	(A) VS. (BCDE) DF = 1	AB DF = 1	CDE DF = 2	(AB) VS. (DE) DF = 1
1	77.04-	27.64	19.24	3.04	101.41†			
2	86.63- -	19.95	14.76	2.40	72.59†			
4	89.03- -	12.63	16.88	2.40	43.30			
6	78.02-	15.73	17.87	3.84	51.40			
8	74.46-	20.58	17.54	7.37	60.21			
10	58.23	21.30	20.22	8.22	60.53			
15	38.37	21.86	23.52	14.93	42.64			
20	43.96	13.98	29.73	7.85	32.37			
25	42.77	24.33	44.73	5.56	80.64	70.76		
30	35.18	39.14	48.58	4.42	143.26	119.72		
35	25.39	74.58	50.64	0.15	186.00	122.50		
40	16.52	65.79	50.89	3.88	251.54†	125.32	2.27	131.03
45	20.71	87.57	51.25	14.79	305.90†	100.49	0.43	248.49†
50	29.16	167.00	57.63	85.44	411.69†	36.83	0.64	629.89‡
55	60.28	277.48‡	55.38	159.98	630.01‡	43.26	5.33	1056.03‡
60	79.37	278.25‡	58.03	169.77	603.68‡	38.81	22.53	1029.14‡
65	99.12	298.52‡	57.14	172.18	677.56‡	52.44	21.10	819.64‡
70	91.95	323.30‡	53.45			142.88	28.42	1093.50‡
75	84.48	313.59‡	43.07			108.90	27.06	1091.34‡
80	70.45	301.80‡	48.00			110.22	52.86	991.25‡
85	66.19	298.46‡	50.36			88.21	52.29	1001.04‡
90	65.04	272.89†	62.99			65.54	62.57	902.87‡

Key:

A—2% procaine + 1:50,000 epinephrine; B—2% Oracaine + 1:50,000 epinephrine; C—2% Xylocaine + 1:100,000 epinephrine; D—2% Kincaine + 1:50,000 epinephrine; E—2% Kincaine + 1:100,000 epinephrine.

- = Indicates the significance of the variance between dogs during the first 8 minutes.

*Comparison among means (Snedecor, G. W.: Statistical Methods, ed. 5, Ames, Iowa, 1956, Iowa State College Press).

†—P<0.05.

‡—P<0.01.

RESULTS—HUMAN

Data obtained and analyzed as in the canine studies are presented in Table IV. When the data used in the construction of Table IV were subjected to analyses of variance, by observation intervals (as in Table III), no statistically significant differences in average threshold increments within the first 20 minutes were revealed.

TABLE IV

AVERAGE THRESHOLD INCREMENTS OF 8 HUMAN SUBJECTS (IN VOLTS) AFTER EACH COMPOUND

MINUTES POST-INJECTION	B	C	D 1:50,000	E 1:100,000
2	11.49	22.55	21.66	15.03
4	17.20	32.67	32.64	26.53
6	18.01	27.78	20.88	32.33
8	22.81	27.71	28.02	39.93
10	22.02	27.56	27.38	38.85
15	21.81	32.55	24.35	24.97
20	0.80	15.31	22.44	14.01
25	0.37	13.34	4.85	13.56
30	0.25	0.96	0.83	11.32
35	0.14	0.39	0.54	0.86
40	0.03	0.20	0.54	0.45

B—2% Oracaine + 1:50,000 epinephrine; C—2% Xylocaine + 1:100,000 epinephrine; D—2% Kincaine + 1:50,000 epinephrine; E—2% Kincaine + 1:100,000 epinephrine.

Table V corresponds to Table II in its construction except that the responses are averages for only the first 15 minutes after infiltration. It is evident that there are two populations in the experimental sample: substantial reactors and modest reactors. It is interesting to note that on Subjects II through VI upper molar teeth were stimulated, while on Subject VII the stimulated tooth was an upper lateral incisor. On Subject VIII, an upper second bicuspid and, on Subject I, an upper molar, with the zygomatic arch relatively distant to the area of infiltration, were stimulated.

TABLE V

AVERAGE THRESHOLD ELEVATION OF EACH HUMAN SUBJECT FOR EACH COMPOUND DURING FIRST 15 MINUTES AFTER INJECTION

SUBJECT	B	C	D 1:50,000	E 1:100,000
I	0.82	65.44	24.83	39.32
II	0.65	1.49	32.52	1.97
III	0.63	0.60	2.99	0.37
IV	0.37	1.11	0.62	0.77
V	0.59	0.48	0.77	44.28
VI	54.26	0.20	0.58	0.42
VII	17.79	71.86	86.00	72.73
VIII	86.00	86.00	71.75	77.00

B—2% Oracaine + 1:50,000 epinephrine; C—2% Xylocaine + 1:100,000 epinephrine; D—2% Kincaine + 1:50,000 epinephrine; E—2% Kincaine + 1:100,000 epinephrine.

If the lesser bone density of Subjects I, VII, and VIII is a legitimate basis of separation, then it becomes important to examine these differences.

Statistically, the average increment after Oracaine was inferior to that of the other three compounds, 6, 8, and 10 minutes after injection in Subjects I, VII, and VIII. No significant differences were evident in the data of Subjects II through VI.

DISCUSSION

Animal experiments in tooth pulp algesimetric comparisons of local anesthetics appear to be sensitive and, using the mental injection, consistent.

Analogous human experiments probably require the same discipline as in dogs; that is, use of the same injection site in all subjects. Furthermore, a larger volume probably should be injected into the human mental foramina than into that of the dogs.

Two per cent Kincaine with either 1:50,000 or 1:100,000 epinephrine and 2 per cent Xylocaine with 1:100,000 epinephrine elicit comparably profound and enduring local anesthesia.

Two per cent Oracaine with 1:50,000 epinephrine and 2 per cent procaine with 1:50,000 epinephrine evoke anesthesia of more brief duration than the other three compounds tested.

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PHASE COMPOSITION OF CROWN AND INLAY PORCELAINS

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DENTAL porcelain may be divided into two major groups according to its use in dentistry. The most common type is that which is used for denture teeth and bridge facings. The second important group comprises the porcelain powders from which crown and inlay restorations are made.

Restorative dentistry has developed special methods for the fabrication of porcelain restorations from the powders.[1] These technics have evolved, often empirically, to control the behavior of the porcelains during forming and firing operations. This behavior is affected by the heterogeneous condition of the porcelains which are made up of different phases. The solid phases in dental porcelains are crystals and glasses.

This investigation was concerned with the identification of phases in crown and inlay porcelains. Physical properties were reviewed as the glassy and crystalline phases were identified in the powders and fired porcelains.

The composition of crown and inlay porcelains has been studied by clinicians who were interested in improving technics for using porcelain in dental restorations. Clark[2] observed sealed pores or blebs in fired porcelains which increased in size and number with increased temperatures. Quartz and amorphous particles have been mentioned by other authors in the dental literature. Photomicrographs of fired porcelains were published by Cohen,[3] Gill,[4] and Klaffenbach[5] to illustrate the effects of various methods of forming and firing.

METHOD OF STUDY

Twelve porcelains were selected from those used routinely in the clinic of the University of Washington School of Dentistry and currently available on the market. They included high, medium, and low firing porcelains of the body, opaque, translucent, and enamel types (Table I).

These porcelains were studied under the Leitz petrographic microscope and crystalline minerals were identified by the usual petrographic technics. The oil immersion method was used to obtain refractive index of the powder particles. Samples of the powders and of the fired porcelains were mounted permanently in Canada balsam. Photomicrographs of typical or exceptional phases were taken on 35 mm. film.

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TABLE I
DENTAL PORCELAINS SELECTED FOR EXAMINATION

PORCELAIN	TYPE	RECOMMENDED TEMPERATURE (DEGREES F.)
Dentsply B	Body, high	2,400
Dentsply 0-4	Opaque	2,400
Dentsply T	Translucent	2,300
S. S. White H-58	Body, high	2,400
Steele's "24" S-4	Body, high	2,400
Steele's "24" S-Y	Opaque	2,400
Steele's "24" S-T	Translucent	2,400
Austenal M-77	Body, medium	2,300
S. S. White M-58	Body, medium	2,300
Apco 16	Body, low	1,875
S. S. White L-58	Body, low	1,700
Pors-on 6	Enamel	1,600

PORCELAIN POWDERS

Ten of the powders were found to be composed of glass particles, some of which contained small mineral grains. Two of the high fusing porcelains contained no glass; instead, they were composed of crushed minerals, predominantly feldspar. In some of the powders, the mineral grains were separate from the glass particles; in most cases, they were enclosed within the glass. The index of refraction of the glass ranged from 1.50 to 1.51. In the Canada balsam mounts, the glass was less than balsam (n = 1.53) and the crystals were higher than the glass. Characteristics of the powder samples are summarized in Table II.

TABLE II
PHASES IN THE PORCELAIN POWDERS

PORCELAIN	PREDOMINANT PHASE	PRINCIPAL MINERAL PHASE	PARTICLE SIZE RANGE IN MICRONS (EXCLUDING FINES)		DISTINGUISHING CHARACTERISTICS
			GLASS	MINERALS	
L-58	Glass	Feldspar	64-32	32-8	Mullite needles 4 to 10 μ in length, normal to the surface of some crystals
16	Glass	Feldspar	80-32	32-16	Small, colored minerals
M-58	Glass	Feldspar	64-32	32-16	Small, colored minerals
M-77	Glass	Feldspar	160-32	32-8	Small, striated minerals
H-58	Glass	Feldspar	80-40	32-16	None
S-4	Glass	Feldspar	96-8	32-8	None
B	Mineral	Feldspar	None	64-8	No glass, some quartz
S-Y	Mineral	Feldspar	64-32	32-8	Some glass
0-4	Mineral	Feldspar	None	64-8	Little or no glass
S-T	Half glass Half mineral	Feldspar	64-32	64-8	None
T	Glass		130-8	32-8	None
6	Half glass Half mineral	Feldspar	96-8	64-32	None

Mineral Phases.—Although the minerals were easily distinguished from the glass, the small size of the particles made identification difficult; fortunately, the twinning which is characteristic of feldspars appeared in many of the grains

under polarized light. Microcline (K Al Si_3O_8), albite (Na Al Si_3O_8), and quartz (Si O_2) were identified from the optical properties of the largest grains, and indices of refraction were determined on minerals not enclosed in glass.[6] Quartz was present in some of the dental porcelains, although in small quantity. It was seen that many smaller feldspar particles did not exhibit twinning.

Very small, colored particles were observed in the body and opaque porcelains. These grains had high indices of refraction and were the only colored particles observed in the porcelain powders under transmitted light. Skinner[7] mentioned the use of spinels in stains, but these particles were too small for positive identification. Different shapes, sizes, and colors were observed; although each sample usually contained only one type. All of them were less than 15 μ (0.015 mm.) in the greatest dimension and all of them displayed high birefringence.

Fig. 1.—Mullite prisms are crystallized in the melted surface of a feldspar particle which is embedded in the glass of a frit powder particle. S. S. White L-58 powder; crossed Nicols, gypsum plate.

An exceptional feature was noted in one of the low fusing body porcelains; this was the presence of mullite needles in feldspar grains within the glass particles (Fig. 1). Mullite crystallizes in melted feldspar as slender prisms which develop in width and in length until they are limited by the size of the feldspar grain. This reaction occurs in white ware bodies at approximately 2,280° F.[8]

Glass Phase.—Many particles of glass did not contain mineral grains and were relatively free from bubbles. Irregular and sharp outlines characterized all the glass particles.

It was established that the principal phases in the dental porcelain powders were feldspars, quartz, and glass; the most predominant was glass. These

phases correspond generally to whiteware bodies. Dental porcelains are similar to commercial porcelain enamels in respect to the short firing time and large amount of frit. A frit is a glass that has been melted, quenched, and ground to the desired fineness. The fritted dental porcelain powders which contained minerals suggested that the fritting process was not carried to a completely glassy state before quenching (Fig. 2). The use of a frit body offers the following advantages in dentistry: (1) melting temperatures are standardized and controlled by the formulation of the frit glass, (2) firing schedules are shortened because the majority of thermal reactions occur in the preparation of the frit, and dental firing consists primarily of re-fusing the glass particles,[7] and (3) the absence of hydrous minerals, such as clay, reduces drying shrinkage and shortens drying time. There are also disadvantages in using this type of porcelain: (1) the frits are difficult to form, and (2) there is no dry strength.

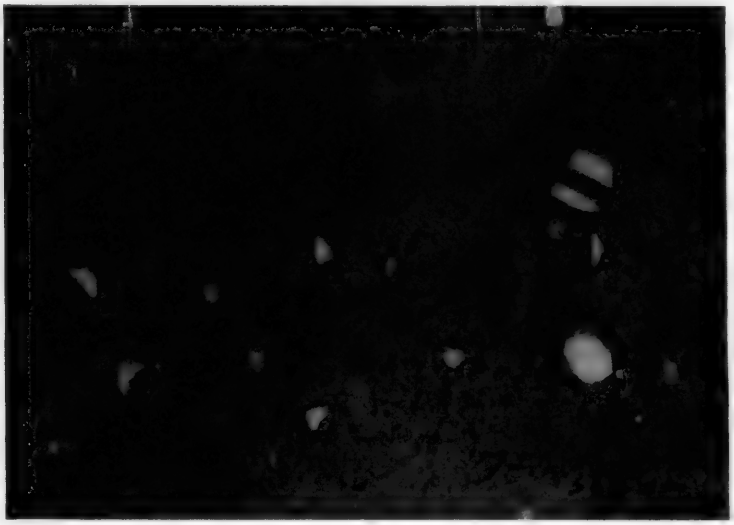

Fig. 2.—The scattered glass particles have dark outlines and the mineral grains are white. Apco 16 powder; crossed Nicols, gypsum plate.

FIRED PORCELAINS

The procedure for forming and firing the porcelain specimen was developed from dental ceramic methods. The equipment included the porcelain carver with serrated handle, a steel mold for forming the porcelain, the dental electric furnace, with pyrometer, and refractory firing trays prepared with platinum foil. The size of the cavity of the mold was 6.5 mm. in diameter, with a depth of 3 mm. The over-all dimensions of the assembled mold were 2 by 1½ by ¾ inches; this size was small enough to use the carver for vibrating the mold. The dental procedure of alternate vibration and blotting was used to compact the porcelain. The powders were mixed with distilled water, condensed in the mold, and then placed on the firing tray for drying.

Each firing consisted of four disks from one powder sample, and 6 minutes were allowed for drying and preheating the samples and firing tray. The maturing temperatures of the porcelains were reached in 8 minutes and were then held for some porcelains up to 12 minutes before the tray was removed from the furnace. Firing tray and samples were cooled to room temperature under a Pyrex dish in approximately 10 minutes.

Because all of the body porcelains were self-glazing, either a bisque or a glazed piece was obtained from the same powder by firing to a lower or higher temperature. In practice, dental restorations are formed by adding successive layers of porcelain powders which are bisque-fired after each addition. These are designated as low, medium, and high biscuit firings. The lower temperatures are used until the desired form has been achieved; then the final glaze-firing of the crown or inlay is made.

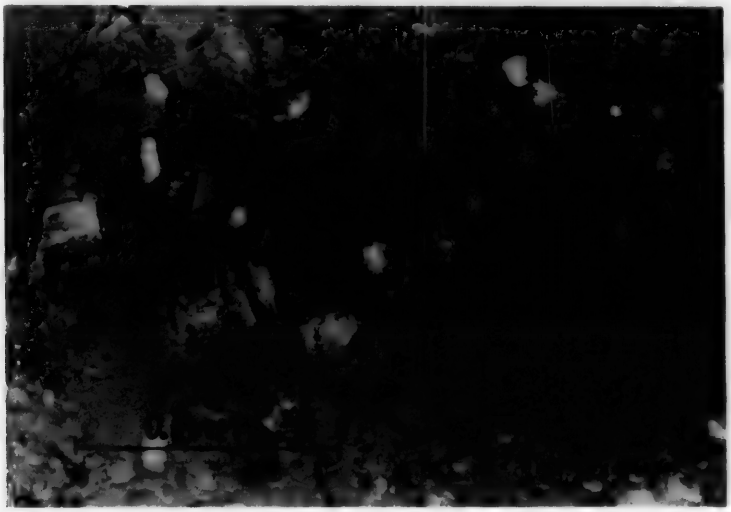

Fig. 3.—Mineral grains are surrounded by glass in the fused frit powder. S. S. White H-58, bisque-fired; crossed Nicols, gypsum plate.

Three types of fired porcelain were produced in this experiment from each of the powders. The bisque was fired 5° to 100° F. below the glaze temperature; the glaze was obtained at the temperature recommended, with or without a soaking period, and the overfired porcelain was produced by a longer soaking time at the glaze temperature.

A microscopic thin section was made from one of the disks from each firing. After bulk reduction was made with a carborundum stone in the dental handpiece, both sides of the section were ground by hand on flat oilstones to under 0.02 mm. thickness. The sections were mounted in liquid Canada balsam for examination under the microscope.

Melting of Mineral Phase.—No new crystal phases were formed when the frit powders were fired (Fig. 3). Melting of feldspars was shown by change in

birefringence, decrease in particle size, and rounding of form. Loss of birefringence denoted changing of the atoms from an organized crystal structure into the random arrangement of glass; however, the high viscosity of feldspar glass hindered its dispersal into the surrounding frit glass, and the shape of the mineral was retained in the glass. Incomplete melting of feldspar crystals resulted in one or more solution rims of feldspar glass which paralleled the rounded outline of a grain whose main body was still birefringent and crystalline. In general, larger grains lost birefringence and became smaller in size and small crystals of feldspar went into solution in the glass matrix. The small, highly colored minerals sometimes became isotropic, but usually they remained unchanged except for rounding in shape. A feature related to firing was fragmentation of feldspar crystals along twin striations.

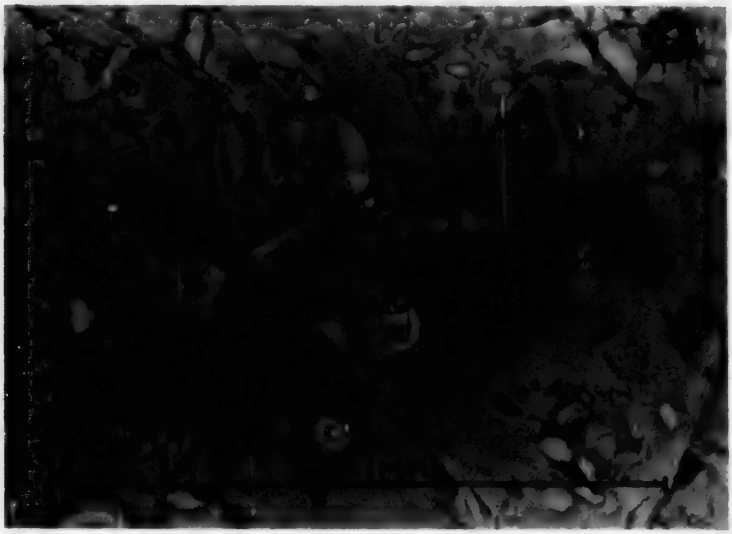

Fig. 4.—Partly melted mineral grains and spherical gas bubbles are embedded in the glass matrix. Dentsply B, glaze-fired; transmitted light.

Melting of Glass Phase.—Glass was the predominant phase in body and translucent porcelains (Fig. 4), and it formed the matrix in fired, opaque porcelains. In the frit porcelain bodies, glass particles were completely fused together in the glaze-firing, but boundary lines were seen in many of the bisque samples at interfaces between melted particles. Some difficulty was experienced in preparing thin sections of these samples because they fractured during grinding.

Melting of feldspar crystals produced areas of feldspar glass which increased the translucency of the porcelain and also helped to stabilize the form during firing. At 2,200° F., ordinary glass has a viscosity of 300 poises; orthoclase glass (feldspar) has a viscosity of 300,000,000 poises, and albite glass has a viscosity of 10,000,000 poises.[8] Solution rims around crystals and striae around bubbles were associated with the high viscosity of feldspar glass and

its slow dispersion into surrounding matrix glass. Similarly, atmospheric gases or gases which formed in the body during firing could not diffuse through the glass, so they were trapped within the glass to form bubbles which increased in size as the gases expanded with further heating.

Bubble Formation.—The usual gas bubbles formed in all porcelains at each firing. In many cases, their development was traced from feldspar grains, which apparently were an additional source of bubbles to those mentioned by Vines, Semmelman, Lee, and Fonvielle.[9] They also appeared in firing translucent porcelains which were almost entirely glass. Bubbles formed as clear areas in the bisque-firing and became spherical and much enlarged upon further firing. The largest bubbles measured in the samples ranged from 0.03 to 0.22 mm.; many were very small when first formed but they expanded with further heating. After the bisque stage, all bubbles were spherical; almost two thirds of the glaze-fired porcelains contained one or more bubbles, approximately 0.10 mm. in diameter.

DISCUSSION

It has been shown that both the powders and the fired products of the dental porcelains consisted primarily of four phases: two types of crystals and two types of glass. The crystal phases were the feldspars and the very small, colored minerals. The glasses were the feldspar glass and the frit glass.

The firing of the porcelains failed to produce any phase different from those already examined in the powders. Thermal behavior of the porcelains corresponded with reactions occurring in normal whiteware bodies at higher temperatures except that mullite was not developed in the dental porcelains because of the short firing time and soaking period, the small particle size of the feldspar,[6] and the small amount present of other reactants which would form mullite. The mullite observed in one of the frits was the product of previous firing. Formation of mullite in ceramic products usually contributes to strength. In this case, however, it was present merely as inclusions in feldspar glass; if an amount sufficient to increase the strength should have developed, it would have increased the opacity of the body porcelains.

The large amount of glass in these dental porcelains suggests that they would have lower resistance to the forces of mastication than would characterize more crystalline porcelains.[10] When the properties of ceramic materials are considered together with the requirements of dental restorations, the use of a crystalline rather than a glass body is indicated. However, such bodies are opaque and bear little resemblance to the appearance of natural enamel. Therefore, to achieve esthetic results, the vitreous, more translucent, porcelain bodies are used in restorative dentistry.

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STUDY OF THE SETTING OF PLASTER

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INTRODUCTION

THIS is a report on the investigation of the volumetric and crystallographic behavior of $CaSO_4 \cdot \frac{1}{2} H_2O$ (i.e., plaster of Paris) when reacted with water or aqueous solutions of various salts. This study could lead to a better understanding of the hydration of this hemihydrate into gypsum, $CaSO_4 \cdot 2H_2O$.

It has long been known that the apparent setting expansion of $CaSO_4 \cdot \frac{1}{2} H_2O$ may be changed by the addition of certain chemicals to the slurry of plaster and water. Sodeau and Gibson[1] demonstrated that K_2SO_4 and several other potassium salts, when added in the proper proportion, will reduce the setting expansion to about 25 per cent of its normal value. No basic explanation of these observations has been given to date.

The final product in the setting of plaster consists of an interlocking mass of gypsum crystals.[2] The setting expansion depends upon the specific volume of the newly formed dihydrate crystals, the size and shape of these crystals, and the nature of the water solution remaining in the crystal mass after the plaster is set.

Van't Hoff and his co-workers[3] have pointed out that the hydration of plaster into gypsum is followed by a contraction of the equivalent volume of the crystals formed. Because of this contradiction, the sum of the volumes of the crystals of hemihydrate and the reacted water is greater than the volume of the resultant dihydrate (gypsum) crystals. Thus, the hydration of plaster of Paris at 33° C. results in a 10.9 per cent contraction in total volume, since, when 1.0 gram of $CaSO_4 \cdot \frac{1}{2} H_2O$ reacts with water, a contraction of 62.49 mm.[3] takes place.

This paper is designed to investigate the factors affecting normal and reduced setting expansion. The dilatometer experiments involve the specific volume of the crystals and the nature of the solution phase. The crystallographic studies are concerned with the effect of morphology on setting expansion.

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MATERIAL AND METHODS

The dilatometer parts *A* and *B* (Fig. 1) were kept in a water bath at a temperature of 30 ± 0.1° C. Before the several parts of the dilatometer were assembled, a weighed amount of $CaSO_4 \cdot \frac{1}{2} H_2O$ was put into flask *A* together with a plastic-covered magnet for stirring. After assembly, the dilatometer was evacuated for 15 minutes, bringing the air pressure down to 10^{-4} mm. Hg. Reservoir *D* was filled with (a) distilled water which had been boiled previously

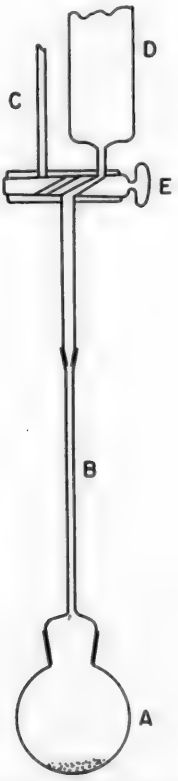

Fig. 1.—Diagram of the dilatometer used in the experiments. Flask *A* has a volume of approximately 50 ml.

to drive off air and equilibrated at 30° C. in the water bath or, (b) salt solution* made from this distilled water. The time when the stopcock *E* was opened was registered as the start of the experiment (time zero). Usually the water was allowed to run in rapidly, thus plaster and water were thoroughly mixed immediately. In some experiments the water was added slowly, so that only a minor part of the plaster was mixed with water at time zero.* After the water had filled *A* and *B*, the upper part of the instrument was removed

*The exact experimental conditions of the dilatometer experiments are given in the legends for Figs. 2, 3, and 4.

from *B*. The water level in *B* was adjusted with a hypodermic syringe and the first reading was taken. Readings of the water level in the capillary *B* were taken at suitable intervals to permit plotting of a smooth curve. The experiments usually were continued until equilibrium was reached (a minimum of 24 hours).

RESULTS AND DISCUSSION OF DILATOMETER EXPERIMENTS

The accuracy of the dilatometer was determined by a series of readings on the volume of pure water. The volume at 30° ± 0.1° C. varied less than 0.3 mm.^{3} during 75 minutes. Thus, any subsequent volume measurements reported have this accuracy limitation. Each curve plotted in Figs. 2, 3, and 4 represents a single series of readings taken periodically under the specific conditions mentioned in the figure legends.

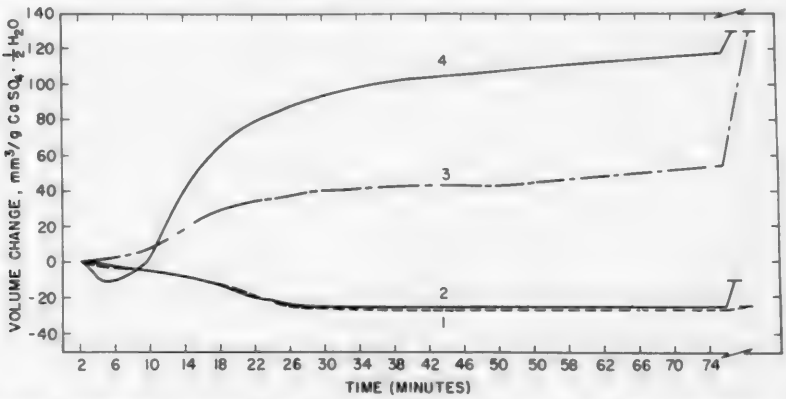

Fig. 2.—Dilatometric curves for mixtures of $CaSO_4 \cdot \frac{1}{2}H_2O$ and distilled water.
Curve 1: **5.0 grams plaster, water added slowly, no mechanical stirring after time zero.**
Curve 2: **2.5 grams plaster, water added slowly, no mechanical stirring after time zero.**
Curve 3: **0.5 gram plaster, water added quickly, no mechanical stirring after time zero.**
Curve 4: **0.5 gram plaster, water added quickly, mechanical stirring during entire reaction.**

A. *Hemihydrate and Water.*—

Fig. 2 shows the results of four dilatometric experiments in which $CaSO_4 \cdot \frac{1}{2} H_2O$ and distilled water were reacted. The volumetric changes are plotted on the ordinate in cubic millimeters per gram of $CaSO_4 \cdot \frac{1}{2} H_2O$, while the time in minutes is plotted on the abscissa. The final volume level, indicated on the right by a line, gives the volumetric change after equilibrium was attained. It is characteristic of all these experiments that the expected contraction of 62.5 mm.^{3} per gram of plaster did not occur. In experiments 1 and 2, a slight contraction did take place, although much smaller than expected, and, in experiments 3 and 4, the final results showed rather pronounced expansions.

The probable explanation of these results follows. When plaster and water are mixed at the beginning of an experiment, a solution is formed which tends to be concentrated with respect to $CaSO_4 \cdot \frac{1}{2} H_2O$. Hemihydrate dissolves very rapidly in water and this initial solution probably has already taken

place when the first reading is taken. At the end of an experiment, when equilibrium has been attained, the water still contains dissolved calcium sulfate, but at this point the concentration is dependent upon the solubility of gypsum.

When calcium sulfate is dissolved in water, there is a volume contraction of the solution. By means of density measurements in a pycnometer it was found that a 0.237 per cent solution of dihydrate in water (below saturation), at 30° C., resulted in a contraction of 0.58 $mm.^3$ per cubic centimeter. The dihydrate is less soluble than the hemihydrate, resulting in a diminished effect upon the volume contraction due to the partial molar volume of the dissolved calcium sulfate. This solubility difference is large enough so that one observes an expansion in solution volume when hemihydrate is converted to dihydrate.

There is another factor involved in the total dilatometer volume change which is dependent upon the solubility difference between hemihydrate and gypsum. Since the former is more soluble than the gypsum, the volume occupied by the undissolved crystals of the final solid (gypsum) will be greater than the volume occupied by the original crystals of hemihydrate at time zero. This works against the volume contraction resulting from the reduced specific volume of the gypsum crystals as compared to the hemihydrate crystals. Both of these factors can be grouped together as the volume change due to the crystal volume differences. The sum of these two effects can be positive or negative, depending upon the factor predominating.

In summary, the total volume change, ΔV_{total}, in the above dilatometer experiments is the sum of (a) ΔV_c, the volume change due to crystal volume differences of the final product when compared with the original solid, and (b) ΔV_s, the volume change due to the differential in partial molar volume of the dissolved sulfate at the end of the experiment. Thus:

$$\Delta V_{total} = \Delta V_c + \Delta V_s.$$

In experiments 1 and 2 (Fig. 2), water was added slowly enough to avoid a thorough mixing of plaster and water and this probably resulted in the formation of a less concentrated solution of calcium sulfate at the start of the experiment than in experiments 3 and 4. The volume increase due to the solubility differential between plaster and gypsum, ΔV_s, would be expected to be smaller than in experiments 3 and 4, where the water at the beginning of the experiment was probably saturated with plaster. The results of experiments 1 and 2 are in agreement with this assumption. The fact that the curve rises to a slightly higher value in experiment 2 than in 1 is probably due to a higher concentration of calcium sulfate at the beginning of the readings in experiment 2.

In experiment 4 (Fig. 2), an initial contraction was observed which did not occur in experiment 3. Both experiments had stirring at "time zero" but 4 had continuous stirring during the reaction, while 3 did not. The initial stirring, as stated above, results in the saturation of the dilatometer liquid with respect to the hemihydrate. If stirring continues, the production of the dihydrate is accelerated and curve 4 can be seen to approach equilibrium at a

faster rate than curve *3*. Note that both achieve the same equilibrium volume at the end of the reaction. This stresses the importance of the initial saturation of the water with hemihydrate in the production of the final volume expansion due to ΔV_s.

The continuous stirring (curve *4*, Fig. 2) resulted in a faster initial precipitation of gypsum with no appreciable change in the concentration of calcium sulfate in the liquid, since the hemihydrate still dominates the solubility at this time. Thus, ΔV_s is minimal within the first 10 minutes of the experiment, while during the same period ΔV_c is appreciable and there is an initial contraction, since ΔV_{total} is negative. Shortly thereafter, ΔV_s becomes more effective than ΔV_c and there is an expansion. In experiment 3, a slight effect of this nature is evident in the slope inflection in the first 15 minutes of the experiment. However, without constant stirring after the initial mixing, the ΔV_s always dominates ΔV_c and the ΔV_{total} is continuously positive.

From the practical view, increased agitation will decrease the setting time of a plaster ($CaSO_4 \cdot \frac{1}{2} H_2O$) slurry. The explanation for this well-known "rule of thumb" is clearly seen in the foregoing experiments, where the production of gypsum is accelerated by stirring. It is not possible to calculate the exact value of ΔV_s (per gram of gypsum produced) as was done with the value for ΔV_c (per gram of hemihydrate converted to gypsum). This value cannot be calculated without an exact knowledge of (a) the solubility of hemihydrate in water, and (b) the change in partial molar volume of calcium sulfate due to changing concentration of solution. If (b) is found experimentally, the value for (a) can be calculated if an experiment can be devised where the exact volume of the dilatometer liquid can be measured at time zero. All the volume values given in this report refer to changes in volume with the first reading taken arbitrarily as the zero volume reading.

B. *Reaction of Hemihydrate in Solutions of K_2SO_4.*—

a. *Dilatometer studies:* The change in volume of a dilatometer liquid as a result of the reaction between $CaSO_4 \cdot \frac{1}{2} H_2O$ and different aqueous K_2SO_4 solutions was recorded. Fig. 3 shows the results for pure water (curve *1*), a 1 per cent K_2SO_4 solution (curve *2*), a 4 per cent K_2SO_4 solution (curve *3*), and a 10 per cent K_2SO_4 solution (curve *4*). All the reactions involving K_2SO_4 are accelerated, as indicated by the rapid rise of the curves in Fig. 3.

Weiser and Moreland[4] claim that the accelerating effect of K_2SO_4 on the setting of plaster is due to an increase in the solubility difference between hemihydrate and dihydrate. This is borne out by the shape of curves *2*, *3*, and *4*, where the effect of an increased ΔV_s is seen in the rapid rise of volume in the first 10 minutes of these reactions. All of the accelerated setting reactions (curves *2*, *3*, and *4*) are changing in volume so rapidly in the early stages that a more exact knowledge of the volume at time zero is needed if the exact volumes at the end of the experiments are to be known. Therefore, any comparison of the final volumes of curves *2*, *3*, and *4* is not significant.

X-ray diffraction studies, pycnometric density measurements, and index of retraction readings (Becke line method) have shown that the equilibrium products of experiments 1, 2, and 3 are pure gypsum ($CaSO_4 \cdot 2H_2O$). The same technics showed the final product of experiment 4 to be a hydrated sulfate of potassium and calcium known as syngenite, $K_2Ca(SO_4)_2 \cdot H_2O$.

When K_2SO_4 is added to a thick $CaSO_4 \cdot \frac{1}{2} H_2O$ slurry (as in a typical casting for dental purposes), a decrease in the normal setting expansion takes place. The equivalent volume of syngenite is 126.8, as compared to 121.7 calculated for the following hypothetical sum: (equiv. vol.) $CaSO_4 \cdot 2H_2O$ – (equiv. vol.) H_2O + (equiv. vol.) K_2SO_4. From these figures one would expect

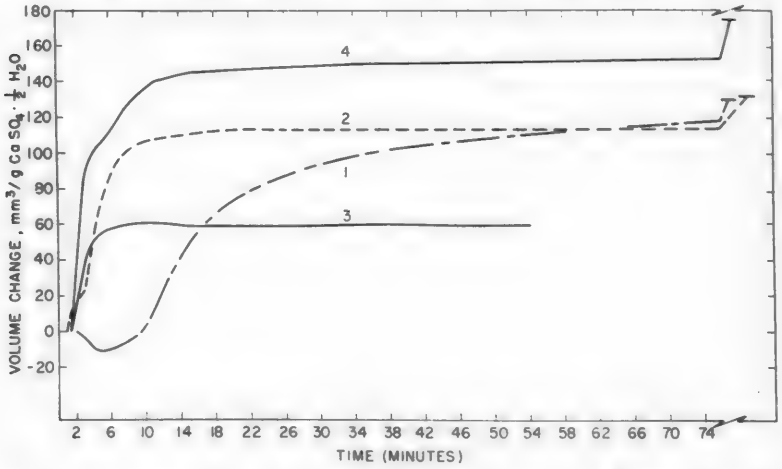

Fig. 3.—Dilatometric curves for mixtures of $CaSO_4 \cdot \frac{1}{2}H_2O$ and different K_2SO_4 aqueous solutions. Dilatometer was filled quickly and mechanical stirring was used throughout all four experiments.

Curve 1: control curve, 0.5 gram plaster and distilled water. (Same as curve 4, Fig. 2.)
Curve 2: 0.5 gram plaster and 1% K_2SO_4 solution.
Curve 3: 0.5 gram plaster and 4% K_2SO_4 solution.
Curve 4: 0.5 gram plaster and 10% K_2SO_4 solution.

an expansion on setting due to a positive ΔV_c. This is corroborated by the expansion shown in each of curves *2, 3,* and *4*. In these experiments, the volume expansion is certainly due to the fact that both ΔV_c and ΔV_s are positive. Another explanation must be sought for the decrease in setting expansion of gypsum when K_2SO_4 is added. Another approach to this problem is reported in Section 4 of this paper.

b. *Solid samples of gypsum:* A series of solid discs of gypsum were prepared by mixing 5 grams of plaster with 5 ml. of K_2SO_4 solutions and allowing the slurries to set between two parallel flat glass plates. The following percentages of K_2SO_4 were used: 2, 4, 5, 6, 7, 8, and 10 per cent. In all cases except 2 per cent, the x-ray diffraction pattern of syngenite was detected. In the dilatometer experiments, above, it was necessary to have between 4 and 10 per cent K_2SO_4 solution before syngenite was formed in the setting of plaster.

The obvious explanation is that, in a thick slurry, water is quickly removed from the reaction in the formation of gypsum, thus enriching the remaining liquid in K_2SO_4.

c. *Plaster and retarding solutions:* The effect of two retarders, borax and gelatin, upon the volumetric behavior of the hydration of plaster was studied by means of the dilatometer. The results (Fig. 4) show that the rate of reaction is markedly decreased in both cases. The reaction between hemihydrate and a 0.5 per cent borax solution (curve *2*) gave a smaller expansion than the reaction between hemihydrate and distilled water (curve *1*). This is probably due to a relatively smaller difference in the calcium sulfate concentration in the dilatometer liquid at the start than at the finish of the experiment. The retarder undoubtedly reduces the rate with which hemihydrate goes into solution

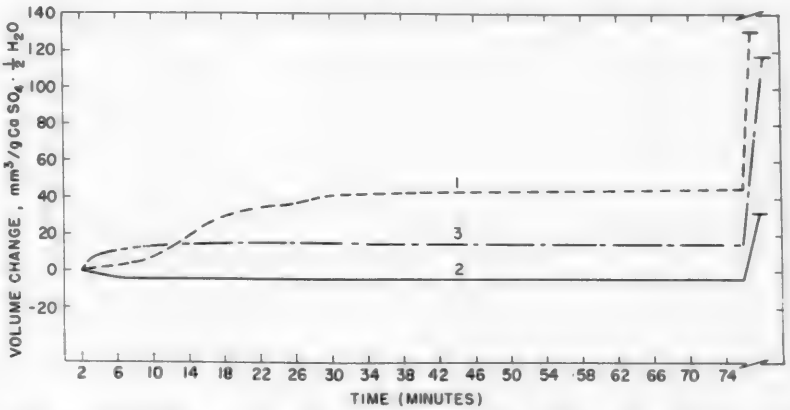

Fig. 4.—Dilatometric curves for mixtures of $CaSO_4 \cdot \frac{1}{2}H_2O$ and retarding solutions. Dilatometer was filled quickly but no mechanical stirring was used in any experiment.
Curve 1: Control curve, 0.5 gram plaster and distilled water. (Same as curve *3*, Fig. 2.)
Curve 2: 0.5 gram plaster and 0.5% borax solution.
Curve 3: 0.5 gram plaster and 0.2% gelatin solution.

just as the lack of stirring did in the experiments with pure water (Fig. 2, curves *1* and *2*). Buchanan and Worner[5] postulated this when they said the retarding effect of borax on plaster setting was due to physically adsorbed borax on hemihydrate crystals resulting in a reduction of solution of the hemihydrate.

The retarding effect of gelatin upon the setting of plaster (Fig. 4, curve *3*) is probably of the same nature as that of borax.[4] The dilatometer curves show the final volume change to be greater for the gelatin than for the borax reaction. Perhaps the borax forms a screening film more quickly and effectively than the gelatin.

CRYSTAL MORPHOLOGY

A series of $CaSO_4 \cdot 2H_2O$ samples was prepared by hydrating 1 gram of plaster of Paris in 100 ml. of different aqueous solutions. Solutions were chosen which had a known effect upon the setting expansion of plaster. Each batch

of $CaSO_4 \cdot \frac{1}{2} H_2O$ and salt solution was stirred magnetically for 24 hours. The resulting gypsum was harvested by filtration and examined in the polarizing microscope for crystal shape.

The setting expansion of plaster is probably due, in great part, to the thrust of the gypsum crystals upon each other during growth. A recent article by Jørgensen[6] presents a summary of this theory. It is reasonable to assume that the tendency for plaster to expand upon setting will change if the crystal morphology changes with a resulting change in the crystal thrust.

The results of this study are shown in Table I. Special attention was given to the changes in the crystal size and shape due to the addition of certain chemicals to the setting water.

TABLE I
CRYSTAL MORPHOLOGY OF GYPSUM FORMED FROM DIFFERENT SETTING LIQUIDS

SOLUTION	EFFECT OF SETTING EXPANSION*	SHAPE OF GYPSUM
Distilled water		Needle-like, very slender
1% K_2SO_4	Pronounced reduction	Needle-like, relatively short and thick
4% K_2SO_4	Pronounced reduction	Needle-like, relatively short and thick
4% KNO_3	Pronounced reduction	Needle-like, relatively short and thick
1% K_2CO_3	Pronounced reduction	Mostly small and spheroid
4% $Al_2(SO_4)_3 \cdot 18H_2O$	None	Needle-like, very slender
4% KCl	Rather pronounced reduction	Needle-like, relatively short and thick
5% Rochelle salt	Pronounced reduction	Very short and massive
4% $Na_2SO_4 \cdot 10H_2O$	Small reduction	Needle-like, relatively short and thick

*According to Sodeau and Gibson,[1] 1927.

The salts which reduce the setting expansion of plaster also change the morphology of the dihydrate crystals in the manner shown above. The only compound which had no effect upon the setting expansion, $Al_2(SO_4)_3 \cdot 18H_2O$, was also found to have no effect upon the crystal shape. This observation on the relationship between the change in crystal habit and the change in setting expansion may be the key to the understanding of the modifying effects of different salts upon the setting expansion of plaster. More work will have to be done in this field to confirm the preliminary results reported herein.

SUMMARY

This study was performed to obtain a better understanding of the setting properties of plaster of Paris, $CaSO_4 \cdot \frac{1}{2} H_2O$. Dilatometer studies have been completed, showing the effect of stirring, accelerators, and retarders on the kinetics of the setting of plaster. Crystal morphology studies show that when plaster is reacted with different salt solutions the crystal habit of the resultant gypsum changes. This change in shape of the final crystals may explain the reduction in setting expansion effected by various additives to the plaster slurry.

The dilatometer experiments exaggerate the contribution of the final water solution to the setting expansion of plaster of Paris. In a set plaster there is

no doubt that the bulk volume will depend principally upon the packing arrangement of the crystals rather than upon the solution effect.

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PHOSPHATASE ACTIVITY IN WHOLE AND PAROTID SALIVA AND ITS RELATIONSHIP TO DENTAL CARIES

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THERE are many reports on phosphatases of body tissues such as blood, bone, and muscle, but comparatively little on phosphatases of saliva. Lura[1] proposed that salivary phosphatase plays a role in the production of dental caries although he did not report any experimental data. Dentay and Rae[2] stated that there was no demonstrable correlation between salivary phosphatase activity and the incidence of caries. Fitzgerald,[3] in initial study of 30 cases, claimed to have found a parallel relationship among acid phosphatase activity, lactobacillus counts and Snyder tests, and suggested a further study of the acid phosphatase activity of saliva as an additional laboratory test for the presence of dental caries. Helman and Mitchell[4] determined the acid phosphatase activity in 49 students, with and without recurrent calculus, and found no significant correlation between lactobacillus counts and phosphatase levels.

In this investigation, the salivary phosphatase activity was measured in 44 students with and without caries. Our results do not support Fitzgerald's conclusion that there is a significant correlation between the level of acid phosphatase activity and the presence of caries.

METHODS

Subjects.—Two groups of dental students were studied ranging from 20 to 25 years of age. One group consisted of 20 students with no evidence of caries or with no more than 3 carious teeth. The other group consisted of 24 subjects, each one possessing 7 or more teeth with caries of the third degree, and several of these students had undergone restorative dentistry or extractions.

Collection of Samples.—About a 2 ml. sample of whole or parotid saliva was collected in a sterile test tube immediately on arising before brushing of teeth, eating, or mouth rinsing. The samples were diluted 5 times with 0.9 per cent sodium chloride and centrifuged at 3,000 r.p.m. for 20 minutes.

Determination of Phosphatase Activity.—Phosphatase activity of saliva was determined essentially according to the method of Shinowara, Jones, and

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Reinhart.[5] Sodium β-glycerophosphate was used as the substrate. The reaction mixture containing 1 ml. of diluted saliva was incubated for 1 hour at 38° C., and was then treated with 30 per cent trichloroacetic acid. After 5 minutes, the sample was filtered and the concentration of inorganic phosphate determined by the method of Fiske and Subbarow.[6] The optical density was measured at a wave length of 340 millimicrons using a Beckman spectrophotometer. The results were expressed in Shinowara-Jones-Reinhart units per 100 ml. saliva.[5]

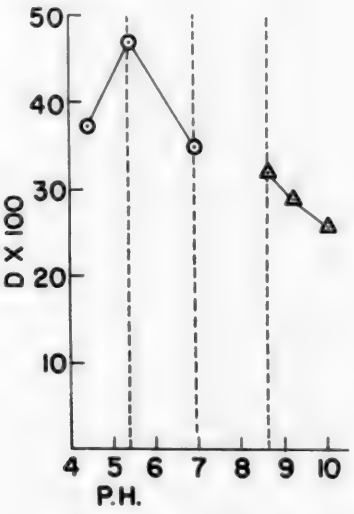

Fig. 1.

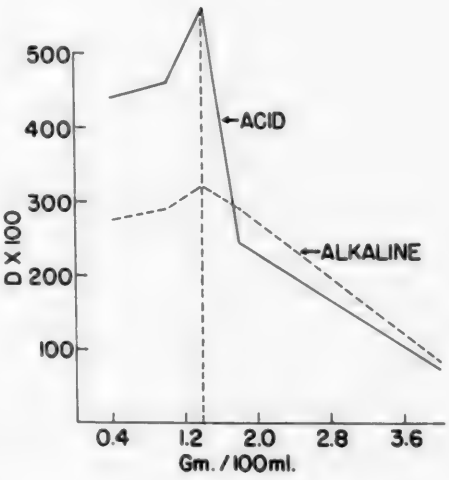

Fig. 2.

Fig. 1.—Effect of pH on phosphatase activity (average of 5 experiments). Note maximal activity at pH 5.4.

Fig. 2.—Effect of concentration of β-glycerophosphate on acid and alkaline phosphatase activity. Note maximal activity at substrate concentration of 1.4 grams per 100 ml.

RESULTS

Optimum pH and Substrate Concentration.—These factors were initially studied since few studies have been made with special reference to salivary phosphatase. The results of a study of the effect of pH on phosphatase activity of saliva are presented in Fig. 1. Maximum activity was obtained at pH 5.4. In subsequent experiments, phosphatase activity at this pH (acid phosphatase) and also at pH 8.6 (alkaline phosphatase) was determined.

An experiment on the effect of substrate concentration is reported in Fig. 2. The assay was carried out at pH 5.4 and at pH 8.6 as described in the preceding section. Under these conditions there was an increase in activity with substrate concentration up to a concentration of β-glycerophosphate of 1.4 grams per 100 ml. The activity decreased at higher substrate concentrations. A concentration of 1.4 grams per 100 ml. was therefore selected for subsequent experiments.

Salivary Phosphatase Activity of Group Without Caries and of Group With Caries.—Table I is a summary of the acid and alkaline phosphatase activities of samples of saliva from 20 students without caries and 24 students with caries at various degrees of severity. The acid phosphatase activity was significantly higher in the control group than in the group with caries, but there was no significant difference in alkaline phosphatase activity between the two groups. The distribution curves for acid phosphatase of the two groups of subjects are presented in Fig. 3. The curves illustrate the great difference in salivary acid phosphatase activities between the two groups.

TABLE I
PHOSPHATASE ACTIVITY (S.J.R. UNITS/100 ML. WHOLE SALIVA) AND DEGREE OF DENTAL CARIES

GROUP WITHOUT CARIES					GROUP WITH CARIES				
SUBJECT NO.	SEX	DEGREE CARIES*	ACID PHOSPHATASE	ALKALI PHOSPHATASE	SUBJECT NO.	SEX	DEGREE CARIES*	ACID PHOSPHATASE	ALKALI PHOSPHATASE
1	F	0	20.8	7.4	1	M	+	6.6	0.9
2	M	–	16.4	4.2	2	F	++	10.4	1.7
3	F	0	23.3	6.3	3	M	++	14.4	2.1
4	M	–	21.2	0.7	4	M	++	12.5	1.0
5	M	–	16.8	1.3	5	M	++	8.4	0.7
6	M	–	12.4	3.2	6	F	++	3.5	1.8
7	F	0	14.3	1.3	7	M	+	8.1	4.2
8	M	0	15.1	0.2	8	M	+	7.6	2.8
9	F	–	15.8	2.0	9	F	++	8.1	1.2
10	M	–	15.6	7.9	10	M	++	2.3	2.3
11	M	0	27.7	3.2	11	M	++	12.5	1.5
12	M	–	15.8	0.4	12	M	++	10.9	1.2
13	M	–	12.1	3.0	13	M	+	4.3	5.6
14	M	0	15.1	0.4	14	M	+	2.5	0.8
15	M	0	26.2	7.2	15	M	+	6.3	3.8
16	M	–	14.9	4.5	16	M	++	1.2	4.5
17	F	–	11.0	2.0	17	M	+	7.2	3.9
18	F	0	24.8	0.5	18	M	+	3.1	0.9
19	F	–	8.9	2.1	19	F	+++	10.8	2.6
20	F	–	6.1	1.2	20	M	++	8.7	0.3
					21	M	++	4.1	2.0
					22	F	+++	8.5	0.7
					23	F	+	1.8	0.3
					24	F	++	1.2	0.2
		Mean†	16.7	2.95				6.9	1.96

*0 = no caries; – = first degree, no more than 3 carious teeth; +, ++, +++ = third degree, grading according to severity of symptoms.

†"t" test for acid phosphatase: $t = 7.34$, $p < 0.005$; alkaline phosphatase: $t = 0.41$, $p > 0.5$.

Daily Variations of Phosphatase Activity.—Since the studies were based on phosphatase determinations of saliva taken on arising in the morning, it was thought of interest to determine the variations in enzymatic activity from day to day. Three students were selected from each group and the acid and alkaline phosphatase activities were determined on arising on 3 consecutive days. Only small variations in activity were found from day to day, the greatest being ± 1.0 Shinowara-Jones-Reinhart units per 100 ml.

Variations Within a 24-Hour Period.—The level of acid phosphatase activity of saliva collected at various times during a 24-hour period was determined in 6 subjects. Two of these experiments are illustrated in Fig. 4. In

the normal subject the acid phosphatase activity was high during the night but decreased rapidly on arising. During the day there was little difference in activity between the control and the subject with caries. At night, when the subjects were sleeping, the acid phosphatase activity of the saliva increased to a very high value in the normal subject but showed a very much smaller increase in the subject with caries.

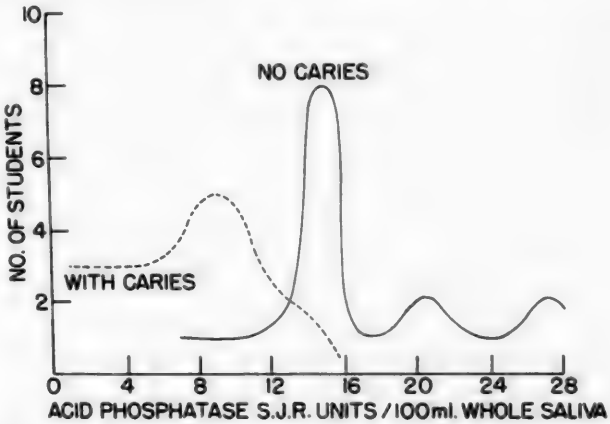

Fig. 3.—Frequency distribution curve of acid phosphatase activity of whole saliva. Note that students without caries have higher levels than those with caries.

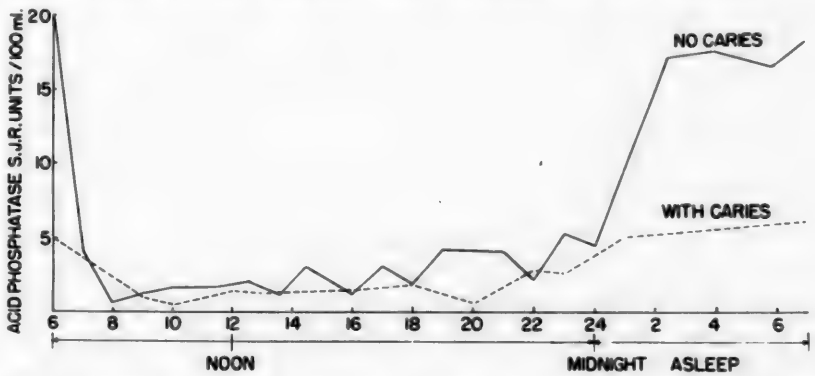

Fig. 4.—Alteration of acid phosphatase activity of whole saliva collected at various times within a 24-hour period. Note higher levels for subject without caries than for subject with caries.

Parotid Saliva.—The origin of salivary phosphatase as described above for whole saliva is still uncertain. The high acid phosphatase activity observed only during sleep and in the early morning may be accounted for by several possible sources from which the enzyme might originate. Among these are: the major and minor salivary glands, bacteria, oral tissues, and ingested substances. Because of these uncertainties, morning samples of whole and parotid gland saliva from each of 12 subjects were collected. The results are recorded in Table II. These studies showed that both acid and

alkaline phosphatase activities of parotid saliva were very low compared to the activity of whole saliva, indicating that the phosphatase enzymes in the whole saliva originate from sources other than the saliva itself. The acid phosphatase activity of whole saliva was much higher in the normal subjects than in those with caries while there was no significant difference between the acid phosphatase activity of parotid saliva from the two groups.

TABLE II
PHOSPHATASE ACTIVITY (S.J.R. UNITS/100 ML.) OF WHOLE AND PAROTID SALIVA

GROUP WITHOUT CARIES					GROUP WITH CARIES				
SUBJECT NO.	ACID PHOSPHATASE		ALKALI PHOSPHATASE		SUBJECT NO.	ACID PHOSPHATASE		ALKALI PHOSPHATASE	
	WHOLE	PAROTID	WHOLE	PAROTID		WHOLE	PAROTID	WHOLE	PAROTID
1	14.1	1.6	1.4	1.6	1	12.5	0.6	1.0	0.7
2	16.4	0.8	4.2	0.8	2	2.3	0.7	2.3	1.1
3	27.7	1.5	3.2	0.8	3	2.2	2.0	1.0	1.6
4	15.8	1.3	1.3	1.3	4	10.5	3.2	2.3	1.2
5	15.6	2.0	7.9	0.2	5	8.4	0.8	0.7	0.5
6	20.8	1.0	7.4	0.5	6	4.3	1.5	5.6	1.0
Mean	18.4	1.4	4.2	0.9		6.7	1.5	2.2	1.0

DISCUSSION

The above experiments demonstrate that there is a significant relationship between the acid phosphatase activity of whole saliva and the presence of caries. The acid phosphatase activity of whole saliva from subjects suffering from caries is, on the average, much lower than the activity of saliva from normal individuals. The acid phosphatase activity of parotid saliva was much lower than that of whole saliva, and its amount was not related to the presence of caries. The high phosphatase activity of whole saliva is therefore exogenous in origin and possibly originates from the bacterial[3] and cellular debris[2] present in native saliva.

Helman and Mitchell[4] investigated lactobacillus counts of 49 senior dental students who were divided into 24 subjects with recurrent dental calculus and 25 normal subjects. They found no significant difference in the phosphatase levels or lactobacillus counts between the two groups. However, they did not state the time of collection of saliva and measured phosphatase activity of the individuals with or without calculus instead of caries. It is not possible to explain the difference between their observations from those reported above.

Fitzgerald's[3] experiments were as follows: Samples of whole saliva from 30 adults were collected and examined for phosphatase activity using disodium p-nitrophenyl phosphate as substrate. The intensity of the yellow color was judged and expressed as 0, +, ++, or +++. He concluded that parallel results were obtained between acid phosphatase activity, Snyder test, and lactobacillus counts of whole saliva. Our own results indicate the opposite, i.e., patients with caries have lower acid phosphatase activity. The reasons for the discrepancy between our results and those of Fitzgerald's are as follows: (a) the possible difference in time of collection of saliva, and (b) the difference in methods of measuring acid phosphatase activity.

Our experiments show conclusively that the difference in phosphatase activity between the normal subjects and those with caries were found only during the night and in the early morning. The influence of the time of day on phosphatase activity of saliva was pointed out by Lura[7] and is clearly evident in our studies. Earlier workers,[2, 3, 4] however, appear to have been unaware of the importance of the time of collection of the sample of saliva and did not indicate in their publication when the samples were collected.

SUMMARY

Acid and alkali phosphatase activities were measured in whole saliva and parotid saliva on arising, in students with and without caries. There was a significant difference in acid phosphatase activity of whole saliva collected from both groups. The students without caries showed a higher activity than those with caries. The alkaline phosphatase activity of the whole saliva was not significantly different between both groups. When parotid saliva was analyzed, there was no correlation between the phosphatase activities and the presence of caries in these students.

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A SOURCE OF ERROR IN ALKALINITY TITRATIONS OF SALIVA

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THE acid-neutralizing capacity of saliva has been the subject of several studies. The major contribution of bicarbonate in this connection has been stressed by Wah Leung[1] among others. The difficulties involved in the titration of the labile bicarbonate have been discussed by Sellman[2] and suitable methods have been developed.[3, 4]

The purpose of the present study is to show how dilution or use of different concentrations of acid influences the titration results, when a bicarbonate system is involved.

MATERIALS AND METHODS

Saliva was titrated undiluted or diluted tenfold, with 0.001 or 0.01 N HCl. Controls consisted of 0.01 M $NaHCO_3$ solutions similarly titrated and also titrations on fresh diluted human serum.

Whole saliva was collected in test tubes at least 2 hours after eating from subjects giving 12 to 18 ml. in a period not exceeding 30 minutes. The aliquots were covered with paraffin oil and stored in a deepfreeze until titrated at room temperature. The bicarbonate controls were freshly prepared 0.01 M $NaHCO_3$ solutions. Freshly drawn human blood was used as a source of serum.

All titrations were performed in 30 ml. beakers and pH readings were obtained with a Beckman model G potentiometer using external glass and calomel electrodes. Samples were measured with 2 ml. tuberculin syringes and mixing during titration was accomplished with a magnetic mixer.

The amounts of acid necessary to reduce pH to 6.5, 6.0, 5.5, 5.0, 4.5, and 4.0 were recorded. In each experiment, 2 to 4 repetitions of titrations were conducted.

RESULTS

The first experiment consisted of titrations of 1 or 2 ml. of saliva from 9 subjects, with 0.01 or 0.001 N HCl and also titration of 1 ml. saliva diluted to 10 ml. with water, with 0.01 N HCl. When 0.01 N HCl was used against undiluted saliva, proportionately more acid was required to reduce the pH to a given point over the range 5.5 to 6.5 than in titrations using 0.001 N HCl or diluted saliva (Table I).

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TABLE I
TITRATION OF DILUTED AND UNDILUTED SALIVA WITH 0.01 AND 0.001 N HCl

	MILLIEQUIVALENTS OF ACID PER LITER SALIVA TO REDUCE pH		
	UNDILUTED SALIVA		DILUTED SALIVA (1:10)
pH ATTAINED	0.001 N HCl	0.01 N HCl	0.01 N HCl
6.5	7.03	8.5	6.0
6.0	9.38	9.9	9.0
5.5	10.76	11.0	10.9
5.0	12.42	12.3	13.0
4.5	14.35	13.9	14.9
4.0	16.46	15.6	17.9

The second experiment consisted of titration of 1 ml. 50 per cent human serum and 10 ml. of 5 per cent serum against 0.01 N HCl. Here, also, it was found that more acid was required to depress pH in the range 6.5 to 5.5 when the sample was undiluted than when it was diluted (Table II).

TABLE II
TITRATION OF DILUTED SERUM WITH 0.01 N HCl

	MILLIEQUIVALENTS OF ACID PER 500 ML. SERUM TO REDUCE pH	
	50 PER CENT SERUM	5 PER CENT SERUM
pH ATTAINED	0.01 N HCl	0.01 N HCl
6.5	15.3	12.7
6.0	19.4	18.0
5.5	22.3	22.2
5.0	25.9	26.1
4.5	30.7	31.2
4.0	38.6	39.1

In a control experiment, bicarbonate diluted and undiluted was titrated with 0.001 or 0.01 N HCl in the same way as were the salivas above. It was found that to reduce pH to a given point in the range 6.5 to 6.0, least acid was required when the sample was diluted and a 0.01 N HCl was used and most acid when the sample was undiluted, again with 0.01 N HCl (Table III).

TABLE III
TITRATION OF 0.01 M AND 0.001 M $NaHCO_3$ WITH 0.01 AND 0.001 N HCl

	MILLIEQUIVALENTS OF ACID PER LITER 0.01 M $NaHCO_3$ TO REDUCE pH		
	0.01 M $NaHCO_3$		0.001 M $NaHCO_3$
pH ATTAINED	0.001 N HCl	0.01 N HCl	0.01 N HCl
6.5	5.70	7.3	5.4
6.0	8.50	9.2	8.1
5.5	9.90	9.8	9.7
5.0	10.50	10.1	10.3
4.5	11.30	10.2	10.8
4.0	13.80	10.6	12.0

To demonstrate that sample size modified the titration results, 2 ml. and 4 ml. samples of 0.01 M $NaHCO_3$ were titrated with 0.01 N HCl. The smaller sample required relatively more acid to depress pH to a given point over the range 5.5 to 6.5 (Table IV).

TABLE IV

TITRATION OF 2 ML. AND 4 ML. SAMPLES OF 0.01 M $NaHCO_3$ WITH 0.01 N HCl

	MILLIEQUIVALENTS OF ACID PER LITER 0.01 M $NaHCO_3$ TO REDUCE pH	
	2 ML. 0.01 M $NaHCO_3$	4 ML. 0.01 M $NaHCO_3$
pH ATTAINED	0.01 N HCl	0.01 N HCl
6.5	8.2	5.6
6.0	9.6	8.4
5.5	10.1	9.8
5.0	10.2	10.2
4.5	10.4	10.4
4.0	10.7	10.7

In a final experiment, 2 ml. samples of 0.01 M $NaHCO_3$ undiluted and diluted with 18 ml. of water were titrated against 0.01 N HCl. Mixing, however, in this case was accomplished by bubbling nitrogen through the samples. Table V shows that under these circumstances there were no significant differences between acid requirement for undiluted and diluted samples in the pH range 5.5 to 6.5. Comparison of the results shown in Tables V and III demonstrates that the use of nitrogen modified the acid requirement upward in both the diluted and undiluted samples.

TABLE V

TITRATION OF 0.01 M AND 0.001 M $NaHCO_3$ WITH 0.01 N HCl WHEN NITROGEN WAS BUBBLED THROUGH

	MILLIEQUIVALENTS OF ACID PER LITER 0.01 M $NaHCO_3$ TO REDUCE pH	
	0.01 M $NaHCO_3$	0.001 M $NaHCO_3$
pH ATTAINED	0.01 N HCl	0.01 N HCl
6.5	9.3	9.1
6.0	9.8	9.8
5.5	9.9	10.0
5.0	10.1	10.3
4.5	10.2	10.6
4.0	10.6	11.7

DISCUSSION

The discrepancy observed above in the amounts of acid required to reduce pH in the range 6.5 to 5.5 is in a direction opposite to that which would be expected, if the effect of dilution is taken into consideration. Further, a series of samples diluted with 0.01 M NaCl behaved identically to those diluted with distilled water. That the phenomenon was not attributable to carbonic anhydrase is indicated by the fact that titrations with serum were essentially the same as those with saliva.

Considering the equation

$$pH = pK + \log. \frac{HCO_3^-}{H_2CO_3} \quad (= \text{physically dissolved } CO_2)$$

an explanation of the observed phenomenon appears to be that in the undiluted samples where the stronger acid was used, the loss of CO_2 through the surface took place rapidly due to a relatively high partial pressure. This explanation is supported by the titration of 0.01 M $NaHCO_3$, in which instance a pattern

corresponding to the one observed in saliva was obtained. By using the above equation, considering pK = 6.2 and the absorption coefficient for CO_2 (Bunsen), it can be calculated that the partial pressure of CO_2 at pH 6.5 in (1) the undiluted sample with 0.01 N HCl is approximately 42 mm. Hg at 20° C.; whereas (2) in the diluted sample, using 0.01 N HCl, the corresponding pressure is 6.4 mm. Hg; and when (3) 0.001 N HCl is used in an undiluted sample, the value is 10 mm. Hg.

If the above explanation is correct, it should be expected that the ratio between volume and surface should be important, and support for this view is provided by the results in Table IV.

If mixing is accomplished by bubbling nitrogen through the samples, the observed discrepancies disappear. The increased amounts of acid required to reduce pH in the range down to 5.5 indicate increased loss of CO_2. That the loss is apparently the same in the diluted and undiluted samples may be explained by considering the increased surface area attributable to the bubbling nitrogen facilitating CO_2 loss.

CONCLUSION

Loss of CO_2 from saliva constitutes a source of error in titrating alkalinity over the range 6.5 to 5.5. At lower pH's, results are modified by dilution of either the sample or the acid. The error resulting from loss of CO_2 can be eliminated by bubbling nitrogen through the saliva, and this procedure can be incorporated usefully in determinations of salivary buffering capacity.

The author wishes to express his appreciation to Dr. James H. Shaw and Dr. John B. Macdonald for assistance and advice in connection with this study.

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OBSERVATIONS ON THE DEVELOPMENT OF OXYTALAN FIBERS IN THE PERIODONTIUM OF MAN

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IN previous reports,[1, 2] a connective tissue fiber in the human periodontal membrane and gingivae has been demonstrated that differs from collagen in staining reactivity. The fiber reacts with aldehyde fuchsin after peracetic acid oxidation. The fibers were also found in tendons, ligaments, the adventitia of blood vessels, the epineurium and perineurium, and surrounding the skin appendages in the dermis. They were not found generally throughout the dermis, in granulation tissue, or in connective tissues of parenchymatous organ stroma. These fibers were subsequently investigated histochemically,[3] were found to be unlike all previously described connective tissue fibers, and were named oxytalan (acid resistant) fibers. Observations devoted to the development of oxytalan fibers are the subject of this report. The development of the fibers was investigated by using a combination of staining methods for acid mucopolysaccharides, collagen, elastic, and oxytalan fibers.

MATERIALS AND METHODS

The material comprised 6 human embryos aged 2 (1), 3 (2), 4 (1), and 6 (2) months, and the upper posterior alveolar processes of 25 individuals ranging in age from 4 months to 79 years. The specimens were fixed in either Lillie's aqueous neutral calcium acetate 10 per cent formalin for 18 to 24 hours at 25° C., or in 95 per cent ethanol for the same time interval. When necessary, ossified tissues were decalcified in 5 per cent formic acid. The specimens were usually cut medially in a mesiodistal direction after decalcification, embedded in paraffin, and sectioned at 6 μ.

Staining Methods for Mucopolysaccharides.—Methods used for the detection of mucopolysaccharides were the dialyzed iron-pierofuchsin method of Rinehart[4]; azure A 0.5 per cent in aqueous phosphate buffer pH 4, 30 minutes, 25° C., periodic acid-Schiff[4]; allochrome[4]; and Gomori's aldehyde fuchsin.[4] The aldehyde fuchsin was aged for 24 hours at 25° C. prior to use, and it was discarded after one week. It was kept in a closed container at room temperature during the week in use.

Staining Methods for Oxytalan and Elastic Fibers.—Methods used for staining elastic fibers were the Taenzer-Unna orcein, Weigert's resorcin fuchsin

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Gomori's aldehyde fuchsin, and Verhoeff's stain as described by Lillie.[4] Oxytalan fibers were stained by the peracetic-aldehyde fuchsin-Halmi,[1, 5] and the peracetic-orcein-Halmi methods.[6]

Method for Protein.—Sections were stained by the alloxan-Schiff method for protein as described by Yasuma and Ichikawa.[7]

Oxidizing Agents and Methods.—Oxidants introduced prior to staining were 0.5 per cent aqueous periodic acid 10 minutes, 25° C., and peracetic acid prepared according to Greenspan,[8] cited by Lillie.[4] Sections were oxidized for 30 minutes at 25° C.

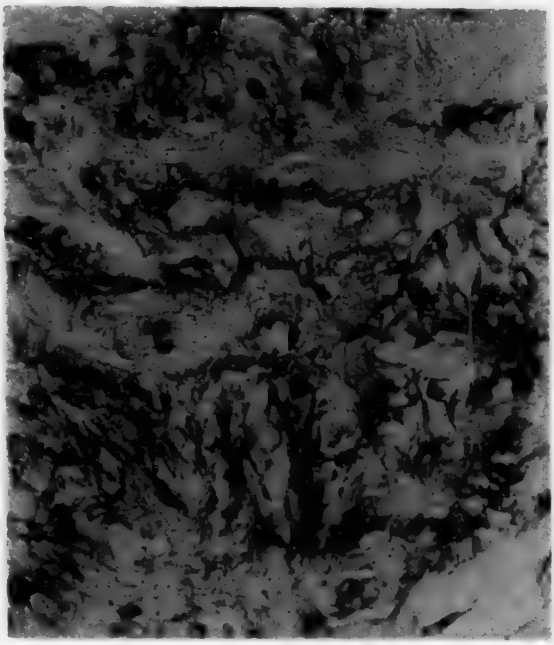

Fig. 1.—Section through connective tissue located immediately occlusal to the developing upper left first molar in a 4-month-old white male infant. Dark staining material is acid mucopolysaccharide staining metachromatically between bundles of collagen which remain unstained. *M*, mucopolysaccharide; *C*, collagen. (Azure A, pH 4, × 540.)

RESULTS

Sections of jaws taken from embryos at various ages revealed that acid mucopolysaccharides and collagen were demonstrated prior to oxytalan fibers. In sections exhibiting the earliest observable stage in the development of oxytalan fibers, stains for acid mucopolysaccharides (azure A, Rinehart, and the peracetic-aldehyde fuchsin-Halmi) reacted strongly between bundles of collagen (Figs. 1 and 2). Additional reactive material between the bundles of collagen may be visualized with the Rinehart stain if sections are oxidized with peracetic acid prior to carrying out the staining procedure. Peracetic acid oxidation increases the amount of orthochromatic but not the metachromatic

substance observed with the azure stain. Since some mucopolysaccharides are identified by the Rinehart stain, and also by the metachromasia they induce with certain basic dyes, it is of interest that a positive reaction is effected by peracetic acid with the Rinehart but not the azure stain. This observation suggests dissimilar mechanisms of reaction of these stains. When sufficient age and development of the embryo and teeth have taken place, oxytalan fibers were demonstrated within the mass of mucopolysaccharide between the bundles of collagen (Figs. 3 and 4). At the time they are first formed, they are

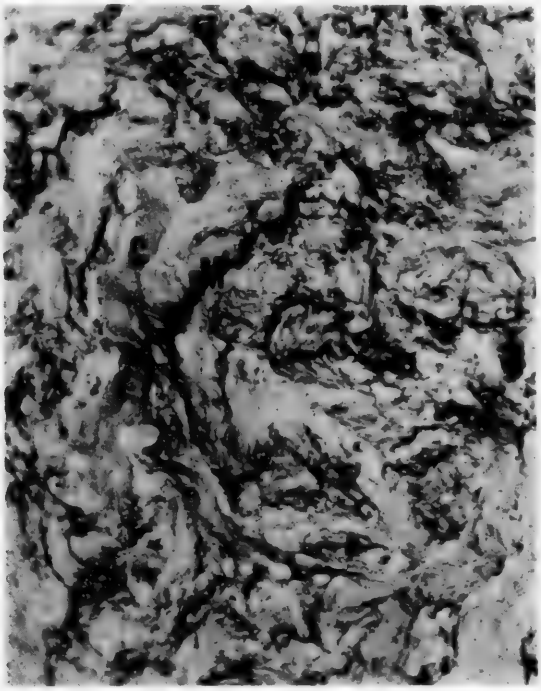

Fig. 2.—Similar section from the same area as Fig. 1. The mucopolysaccharide stains dark purple between bundles of collagen which stain green with the counterstain. (Peracetic-aldehyde fuchsin-Halmi stain, × 540.)

demonstrated by the peracetic-aldehyde fuchsin-Halmi or the peracetic-orcein-Halmi stains. The mucopolysaccharide surrounding the fibers is demonstrable with the azure A, Rinehart, peracetic-Rinehart, peracetic-aldehyde fuchsin-Halmi, but not peracetic-orcein-Halmi, orcein, or any of the other elastic tissue stains tested. Oxytalan fibers are not revealed by the azure or Rinehart stains with or without previous oxidation. Sections stained with the alloxan-Schiff method for proteins revealed no further information.

Oxytalan fibers develop in two locations in developing jaws. They develop (1) adjacent and peripheral to the outer enamel epithelium, and (2) in the oral mucosa superior (in the lower jaw) and inferior (in the upper jaw) to

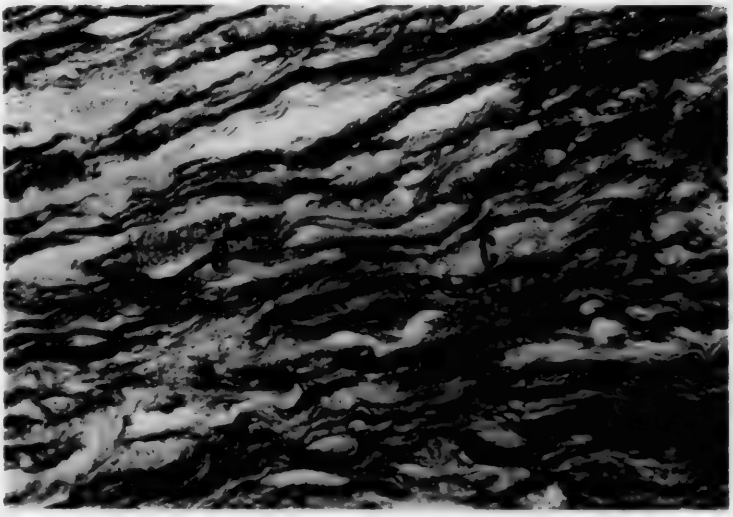

Fig. 3.—Section through connective tissue located immediately occlusal to the developing upper left first deciduous molar in a 4-month-old Negro female infant. Dark staining material is principally oxytalan fibers that appear to develop out of mucopolysaccharide between bundles of collagen. At this stage, some of these oxytalan fibers will also stain with orcein if they are previously oxidized with peracetic acid. *O*, oxytalan fibers. (Peracetic-aldehyde fuchsin-Halmi stain, × 540.)

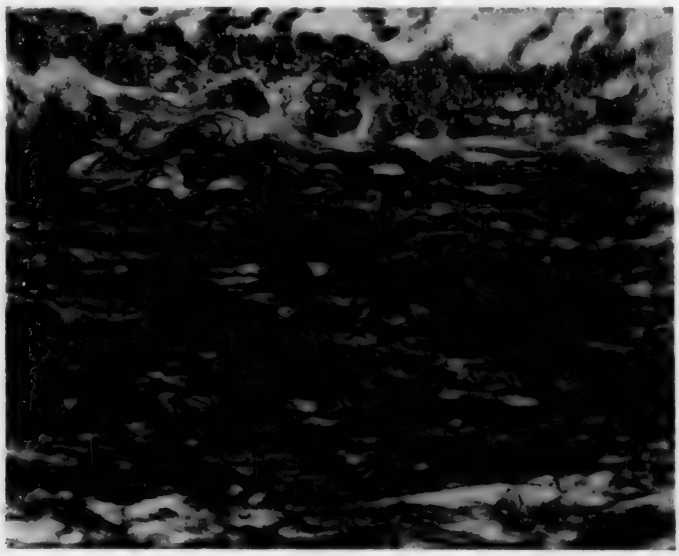

Fig. 4.—Another area from the same tooth as Fig. 3 showing development of oxytalan fibers lateral to the enamel epithelium. *E*, enamel epithelium. (Peracetic-aldehyde fuchsin-Halmi stain, × 540.)

the developing teeth. The first group become fibers of the periodontal membrane or gingivae, and the second group become fibers of the gingivae. The fibers in the oral mucosa develop first and are seen in the embryo at approximately 6 months of age. The age that the second group appears is related to the stage of development of the teeth. Oxytalan fibers arising peripheral to

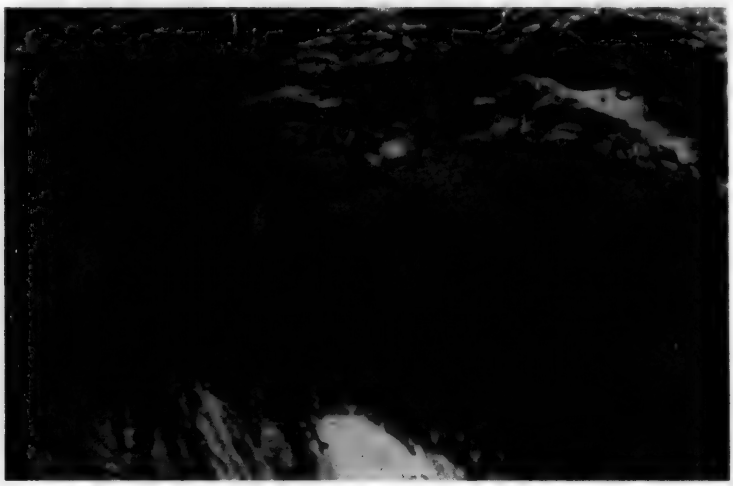

Fig. 5.—Section from the upper left developing deciduous first molar of a 1-year-old white female infant demonstrating incorporation of oxytalan and collagen fibers into the cementum at the site where Hertwig's epithelial root sheath fragments. *H*, cells of Hertwig's sheath; *D*, dentin; *K*, fibers of von Korff. (Peracetic-aldehyde fuchsin-Halmi stain, × 1625.)

Fig. 6.—Same as Fig. 5. (× 355.)

the tooth buds were not found in any sections of the embryonic jaws that we examined; presumably, they arise at an age older than any embryo investigated, since they were found surrounding the outer enamel epithelium of developing teeth of infants. It is to be remembered that we had no embryos older than 6 months. We obtained our best demonstration of developing oxytalan fibers (in the second location) adjacent to the developing first and second molars from

a 4-month-old infant. At this age, there is approximately 0.5 mm. of dentin on the occlusal surface of the second deciduous molar, and about 0.75 mm. of dentin on the occlusal surface of the first deciduous molar. On the first molar, dentin extends laterally almost to the cementoenamel junction. In the connective tissues surrounding the outer enamel epithelium, and in the oral mucosa superior to the developing teeth oxytalan fibers are seen in various stages of development. In general, the fibers in the oral mucosa are larger than those developing around the teeth.

Fig. 7.—Section of periodontal membrane extending distally from the middle third of the distal-buccal root of the upper left deciduous molar from a monkey with a mixed dentition. An arrow points to an oxytalan fiber that has one end attached to a lymphatic vessel and the other end embedded in the cementum of the tooth. (Peracetic-aldehyde fuchsin-Halmi stain, × 270.)

Oxytalan fibers in the developing oral mucosa are oriented mostly in a plane parallel with the mucosa. After eruption of the teeth, they are seen in the gingivae circumscribing the teeth as well as following the course of the collagen fibers in the free gingivae.

Oxytalan fibers that develop lateral to the developing tooth buds remain close to the outer enamel epithelium. With further maturation of the teeth, they develop and proliferate lateral to Hertwig's epithelial root sheath as it progresses apically. Within a short time after Hertwig's epithelial root sheath has progressed apically, influenced odontoblasts on its inner surface have undergone differentiation and dentin is produced. Hertwig's epithelial root

sheath undergoes disintegration as soon as dentin is produced adjacent to it. At the site where Hertwig's epithelial root sheath fragments, cementogenesis occurs, and collagen and oxytalan fibers are observed to be embedded within the cementum (Figs. 5 and 6). It is evident from these observations that oxytalan fibers are first inserted in the cementum at the cementoenamel junction, and are then continually embedded in the cementum as it is produced, ending at the apex of the tooth.

With further development, and after functional demands are placed upon the teeth, oxytalan fibers undergo a rearrangement and increase in size. When oxytalan fibers are first seen, they are of a size approaching the limit of resolution of the light microscope (less than 0.5 micron in diameter). In the adult, particularly in the transeptal area where considerable mechanical stresses occur, oxytalan fibers are larger than when first formed, and larger than in any other area about the tooth. In one case in which the teeth were used as bridge abutments, oxytalan fibers in the transeptal area were increased both in size and number above normal.

As the vascular system becomes established in the periodontium, some oxytalan fibers extend from the adventitia and become attached to the teeth (Fig. 7). Many of the vessels are in the apical half of the periodontium. Oxytalan fibers about these vessels progress in an occlusal direction, frequently branch, and the branches are cemented into the tooth. The distribution of oxytalan fibers will be the subject of a subsequent report in preparation.

DISCUSSION

Oxytalan fibers appear to develop out of a mass of mucopolysaccharide between bundles of collagen. The mucopolysaccharide is visualized by the azure A, Rinehart, peracetic Rinehart, and the peracetic-aldehyde fuchsin-Halmi stains. However, it is interesting that after the fiber is formed and is visualized by the peracetic-aldehyde fuchsin-Halmi stain, it cannot be seen by the Rinehart or azure stains with or without previous oxidations. It is suggested that a degree of maturation apparently takes place whereby mucopolysaccharide may combine with protein or other substances in such a fashion that the reactive groups required for the azure and Rinehart stains are used. In addition, at this time other reactive groups are formed that now react with orcein after peracetic acid oxidation. It is a curious fact that orcein does not react with the mucopolysaccharide with or without previous peracetic acid oxidation.

The observations that only very small oxytalan fibers were found in sections from embryos, and that only very small oxytalan fibers were seen to be initially embedded in the cementum are submitted as evidence that these fibers increase in size, since oxytalan fibers, especially in the transeptal area in functional deciduous teeth, are several times the size of those initially present (Fig. 8). It is tempting to postulate that stress placed upon the fibers is primarily responsible for the increase in size of the fibers. However, oxytalan fibers develop and apparently increase in size in the oral mucosa of embryos and infants prior to the application of any known mechanical stress.

Another unexplained observation was the strong reactivity of stains for acid mucopolysaccharide between bundles of collagen in the gingivae and oral mucosa of some adults. The mucopolysaccharide had the same staining characteristics as that which surrounded oxytalan fibers during their development and yet, no fibers were demonstrable. In this respect, the gingival structures are unlike skin, whereby elastic and collagen fibers are distributed rather uniformly throughout, along with small amounts of mucopolysaccharide. In the gingivae, bundles of collagen may be present with oxytalan fibers, or bundles of collagen may be surrounded by mucopolysaccharide without any other demonstrable fibrous element.

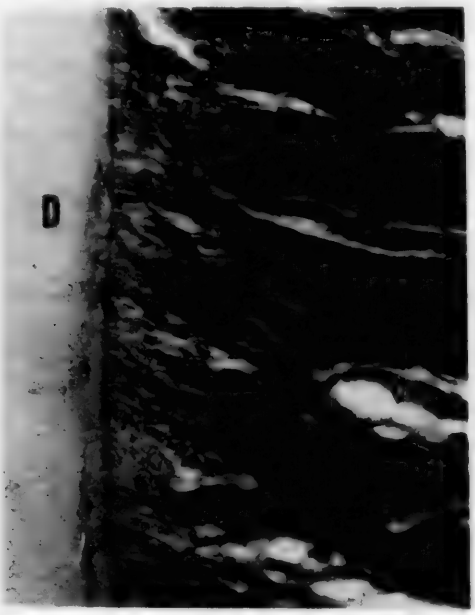

Fig. 8.—Section from the upper left deciduous first molar and periodontal membrane of a 3-year-old white male near the cementoenamel junction demonstrating larger oxytalan fibers at this age than when initially formed and embedded into the cementum. Arrows point to oxytalan fibers. (Peracetic-aldehyde fuchsin-Halmi stain, × 530.)

Throughout the course of these studies it was observed that peracetic acid oxidation of sections greatly increased the amount of reactive material with the Hale (Rinehart) stain. In sections of skin, greatly increased amounts of reactive materials were seen between bundles of collagen, surrounding elastic fibers, and surrounding collagen fibers to a lesser degree. The amount of stainable material steadily decreased in sections of skin from individuals with increasing age. This material remained orthochromatic with the azure A stain in sections oxidized with peracetic acid. The periodic acid-Schiff reaction stains a moderate amount of material rather generally throughout the dermis, and for this reason the effect of peracetic acid oxidation on the material revealed by the periodic acid-Schiff stain is difficult to assess. Since the reactive

material visualized with the peracetic-Rinehart stain encompasses developing elastic, oxytalan and collagen fibers, and decreases in amount with increasing age, its use in the construction of these various fibers is suggested. It is possible that the material demonstrated by this method is similar to that visualized by sulfation procedures.[10, 11]

SUMMARY

The development of oxytalan fibers was investigated with the use of staining methods for mucopolysaccharides, elastic, collagen and oxytalan fibers. Oxytalan fibers appear to develop from a mass of mucopolysaccharide between bundles of collagen, and are first demonstrable in the oral mucosa of embryos at approximately 6 months of age. They develop in the periodontal membrane as Hertwig's epithelial root sheath proliferates apically, and are inserted along with collagen in the cementum as Hertwig's sheath fragments. They apparently increase in size under certain conditions.

The author acknowledges the technical assistance of Mr. Clifton Link, Jr.

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THE EFFECT OF PEPTIDES AND AMINO ACID DERIVATIVES ON ACID FORMATION IN SUGAR-SALIVA MIXTURES

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INTRODUCTION

THE reduction in microbial acid formation on the tooth surface can be accomplished chemically on at least two levels of organization: first, at the enzyme level,[1] and second, at the microorganism level. The first approach is specific; one or more enzymes within the microorganisms are inhibited in such a manner that acid production either is diminished or prevented. The second approach, much less specific, attempts to stop the growth of the microorganism and thereby stop its acid production. It would seem best, for a number of reasons, to stop or reduce acid formation with the least amount of upset to the existing oral microbiota. Accordingly, the more specific approach appears to possess certain advantages. Furthermore, as suggested earlier,[2] one practical approach to the problem of introduction of the inhibitor compound into the mouth would be its incorporation into the dietary sugar. This would allow the inhibitor to be present on the tooth surface at its highest concentration at the same time that the sugar concentration is the highest.

Many compounds with inhibitor properties would be unsuited for such an application because of their toxicity. However, with the above considerations in mind, one group of substances that appeared promising was peptides and other amino acid derivatives. Most of them should not be toxic to the human being; instead they should be utilized, in the form of their constituent amino acids, as food material following digestive hydrolysis. Over a period of years, as they have become available from numerous sources, such compounds have been tested for acid inhibition in this laboratory. The present paper contains the results of such tests.

METHODS

Testing of the compounds for acid inhibiting action was accomplished by the routine procedure which has evolved in this laboratory.[3-7] The test involves the 4-hour incubation, at 37° C., of vials containing 10 ml. aliquots from a pooled sample of fresh caries-active saliva plus 0.1 gram quantities each of glucose and tricalcium phosphate. The compounds to be tested are added in

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TABLE I

EFFECT OF COMPOUNDS ON ACID FORMATION IN SUGAR-SALIVA MIXTURES

COMPOUND*	AMOUNT MG./100 ML. OF SALIVA	PER CENT REDUCTION IN ACID FORMATION	COMPOUND	AMOUNT MG./100 ML. OF SALIVA	PER CENT REDUCTION IN ACID FORMATION
			True Peptides		
glycylglycine	50	74	glycyl-L-asparagine	50	96
	10	57		10	22
glycyl-DL-alanine	50	94	glycyl-D-asparagine	50	34
	10	66			
glycyl-DL-valine	50	80	glycyl-L-tryptophan	50	0
	10	62			
glycyl-DL-leucine	50	88	DL-alanylglycine	50	82
	10	79		10	75
	1	20	DL-alanyl-DL-alanine	50	100
glycyl-DL-isoleucine	50	54		10	68
	10	26	DL-alanyl-DL-valine	50	60
glycyl-DL-α-amino-butyric acid	50	63		10	29
	10	0	L-alanyl-DL-isoleucine	50	50
glycyl-DL-methionine	50	81	L-alanyl-L-leucine	50	34
	10	0	DL-alanyl-DL-norvaline	50	91
glycyl-DL-serine	50	54		10	40
glycyl-L-tyrosine	50	55	DL-alanyl-DL-methionine	50	100
	10	41		10	84
glycyl-DL-phenylalanine	50	75		1	32
	10	57	DL-alanyl-DL-phenylalanine	50	82
L-leucylglycine	50	91		10	54
	10	0	L-phenylalanyl-glycine	50	70
D-leucylglycine	50	74		10	46
	10	59	DL-phenylalanyl-glycine	50	87
DL-leucylglycine	50	72		10	0
	10	0	DL-histidyl-DL-histidine	50	58
L-leucyl-L-alanine	50	64	DL-methionylglycine	50	83
	10	59		10	17
L-leucyl-L-leucine	50	72	L-prolylglycine	50	53
	10	60		10	71
L-leucyl-L-phenylalanine	50	53	glycylglycyl-glycine	50	50
D-leucyl-L-tyrosine	50	29	DL-alanylglycyl-glycine	50	67
L-leucyl-L-tyrosine	50	52		10	33
L-leucyl-L-tryptophan	50	0	D-leucylglycyl-glycine	50	47
DL-valylglycine	50	42	L-leucylglycyl-glycine	50	68
DL-isoleucyl-glycine	50	65		10	35
	10	0	DL-leucylglycyl-glycine	50	100
				10	63

*All compounds listed in this table were obtained from Mann Research Laboratories.

small quantities, one to each vial, and the results compared with a control vial to which no test compound was added. In the control vial, salivary microorganisms ferment glucose to acids, the pH drops and, at a sufficiently low pH, the suspended tricalcium phosphate begins to dissolve. In the other vials, less acid formation and hence less calcium dissolution results when the test compound has inhibitor activity. Calcium determinations are made on the pooled saliva sample without incubation, upon the incubated blank saliva sample, and upon the incubated saliva sample with test compounds added. The increase in calcium concentration in the test vial divided by that in the control vial yields the fraction of inhibition provided by the compound. Results are reported in

TABLE II
EFFECT OF COMPOUNDS ON ACID FORMATION IN SUGAR-SALIVA MIXTURES

COMPOUND	SOURCE	AMOUNT MG./100 ML. OF SALIVA	PER CENT REDUCTION IN ACID FORMATION
	Polyamino Acids		
Poly-DL-alanine	Polaroid Corp.	50	100
		10	0
Poly-γ-methyl-glutamate	Polaroid Corp.	50	55
		10	0
Poly-L-glutamic acid	Stahmann*	50	14
		10	43
Poly-L-aspartic acid (n = 22)	Katchalski†	50	0
Poly-L-tyrosine (n = 28)	Katchalski	50	0
Poly-L-lysine (n = 31)	Katchalski	50	100
		10	36
Poly-L-lysine	Stahmann	50	100
		10	100
		1	5
	Natural Polypeptides		
Crude protamine nucleinate	Mann Research Lab.	50	50
		10	41
Crude protamine nucleinate (different sample)	Mann Research Lab.	25	92
Salmine	Mann Research Lab.	50	45
		10	8
Spermine hydrochloride	Mann Research Lab.	50	100
		25	100
		10	79
		5	88
		2.5	68
		1	0
Spermidine phosphate	Mann Research Lab.	50	94
		25	100
		10	94
		5	28
		2.5	17
Crude corticotropin (lot 267)	Armour Laboratories, Inc.	50	0

*Kindly furnished by Dr. Mark Stahmann, University of Wisconsin, Madison, Wis.
†Kindly furnished by Dr. Ephriam Katchalski, Hebrew University, Jerusalem.

per cent inhibition. Compounds generally are tested initially at a level of 50 mg. per 100 ml. saliva (5 mg. in the vial with the 10 ml. saliva sample). Those showing good inhibition are tested at lower levels in subsequent tests.

RESULTS

The accompanying tables summarize the results obtained. To provide an orderly presentation the compounds studied have been grouped in the tables into true peptides, polyamino acids, natural polypeptides, amino acid derivatives, and amino acid anhydrides. It will be noted that a number of the compounds inhibited acid formation. Those which showed appreciable inhibition at levels below 50 mg. per 100 ml. of saliva are worthy of further attention. For example, several dipeptides containing a glycyl moiety showed good inhibition at the 10 mg. level; likewise, some alanyl dipeptides were active. Only

TABLE III

EFFECT OF COMPOUNDS ON ACID FORMATION IN SUGAR-SALIVA MIXTURES

COMPOUND	SOURCE	AMOUNT MG./100 ML. OF SALIVA	PER CENT REDUCTION IN ACID FORMATION
	Amino Acid Derivatives		
γ-Aminobutyric acid	General Mills, Inc.	50	0
L-Homoarginine	General Mills, Inc.	50	12
L-Homocitrulline	General Mills, Inc.	50	21
3-Monoiodo-L-tyrosine	Mann Research Lab.	50	40
Cycloserine	Mann Research Lab.	50	52
		10	10
Glycine ethyl ester HCl	N. U.: Wachtl*	50	59
		10	49
N-phenylglycine	Eastman	50	100
		25	0
N-phenylglycine ethyl ester	Eastman	50	56
γ-methyl-L-glutamate	General Mills, Inc.	50	0
γ-ethyl-L-glutamate	General Mills, Inc.	50	7
Copper complex of γ-methyl-L-glutamate	General Mills, Inc.	50	100
		25	100
		10	100
		1	43
L-glutamyl hydrazide	General Mills, Inc.	50	92
		25	47
L-arginine carbonate	General Mills, Inc.	50	0
tri-L-argininium citrate	General Mills, Inc.	50	0
tri-L-argininium fumarate	General Mills, Inc.	50	0
L-arginine safflower fatty acid salt	General Mills, Inc.	50	27
L-arginine-pyrrolidone carboxylic acid	General Mills, Inc.	50	34
L-lysine safflower fatty acid salt	General Mills, Inc.	50	36
L-lysine-L-glutamic acid monohydrate	General Mills, Inc.	50	17
Pyrrolidone carboxylic acid	General Mills, Inc.	50	12
	Amino Acid Anhydrides		
Glycine anhydride	N. U.: Wachtl*	50	68
Alanine anhydride	N. U.: Wachtl*	50	75
Leucine anhydride	N. U.: Wachtl*	50	85
Sarcosine anhydride	N. U.: Wachtl*	50	100
		10	94
		1	51

*Synthesized in this laboratory by Dr. Carl Wachtl.

polylysine among the polyamino acids showed good activity. Some of the crude natural polypeptides appear promising. None of the compounds listed in Table III, under Amino Acid Derivatives, appears to be of practical importance. Of the anhydrides, sarcosine anhydride showed good inhibition.

DISCUSSION

Inhibition of acid formation at the 50 mg. per 100 ml. level is not of practical importance; that rather high concentration simply serves as a preliminary

screening level. Those compounds showing promise are tested at successively lower concentrations. Actually, the buffering capacity of the peptides at the 50 mg. per 100 ml. level should be appreciable. Therefore, a part of the apparent reduction in acid formation shown by peptides at that concentration level might be expected to result from neutralization of acid formed. Apart from this, however, a more specific effect was operating to inhibit acid formation as shown by the variation in amount of acid inhibition exhibited by the various peptides at the 50 mg. level. Furthermore, numerous peptides showed appreciable inhibition at lower concentrations where their buffering action would be negligible. In addition, it was noted that peptides identical except for D- and L-configuration differed in acid inhibition.

To be of commercial significance as an inhibitor to be incorporated into sugar-containing foods, a compound probably would need to be active at, or below, the 5 mg. per 100 ml. level. It must, in addition, be relatively inexpensive. Although none of the compounds tested appear to meet these requirements, there was sufficient activity to encourage further work.

The finding that several dipeptides showed inhibitor action is of special interest. These compounds represent typical protein degradation intermediates and should exhibit no toxicity in the gastrointestinal tract. Furthermore, compounds of this type would be produced in the mouth during putrefaction. The fact that they inhibit acid formation, therefore, is of considerable interest from two standpoints. First, peptides may play a role in the frequently mentioned antagonism between putrefaction and fermentation; second, peptides may be found which will serve as good inhibitors for practical use as just outlined.

The results obtained suggest several facets requiring further attention. It will be necessary to (1) test other peptides as they become available; (2) test mixtures of peptides to detect any cumulative or synergistic action; (3) test fractions of peptides isolated from partial hydrolysates of proteins; and (4) synthesize and test peptides which would appear to have the greatest promise on the basis of the other information accumulated.

SUMMARY

Compounds containing amino acids were tested for their activity in diminishing the amount of glucose degradation to acid in saliva. Several peptides were found to exhibit appreciable activity in the concentration level at or below 10 mg. per 100 ml. of saliva. These results suggest at least two possibilities pertaining to dental caries. First, it is possible that peptides arising from putrefaction in the mouth might accumulate on the tooth surface in sufficient concentration to diminish acid fermentation. Second, although none of those tested appear sufficiently active, it may be possible to find peptides with the requisite inhibitor activity to be of practical importance.

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DENTAL CARIES IN THE SYRIAN HAMSTER

VIII. The Induction of Rampant Caries Activity in Albino and Golden Animals

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SYRIAN hamsters are useful in experimental caries studies not only because lesions can be induced in their molar teeth within a short period of time but also because it is possible to see and to photograph many of the lesions as they develop in the live animal[1, 2] (Fig. 1). In the past 3 years, over 1,000 golden and 850 albino hamsters have been raised and studied. This report covers the methods now employed to induce rampant dental caries within a 35- to 42-day period and briefly describes the pattern of caries now observed.

EXPERIMENTAL

Golden and albino hamsters of known "susceptibility" are used for the production of litters which are used for experimental assays. All animals used as breeders are fed a stock diet composed of equal parts of laboratory chow* and a rabbit diet† which has been pulverized by passage through a Hobart meat grinder. Supplements of fresh apples, carrots, and kale are given twice a week to stock females and daily to pregnant and nursing animals.‡

Animals are weaned when their weights are between 25 and 30 grams (approximately 20 days of age). Newly weaned animals are grouped together with 3 to 5 animals in a cage. Males are generally not separated from females while they are fed the test diet which is composed of confectionery sugar 59%; skim milk powder 27%; whole wheat flour 6%; alfalfa powder 3%; liver powder (1:20, Wilson) 2%; whole liver powder 2%; salt mixture 1%.§ Distilled water is always available, and supplements of fresh apples, carrots, and kale are supplied once a week. The usual procedure has been to run golden hamsters for 42 days on this regimen and albino animals for 35 days. At this time the animals are examined without anesthesia and are tentatively scored. However, this score is not accurate because plaque material often covers much of the

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*Purina Laboratory Chow (meal form). Ralston Purina Company, St. Louis Mo.

†BB Laboratory Rabbit Diet. Maritime Milling Company, Buffalo, N. Y.

‡Rats should not be kept in the same room with breeding hamsters as their presence is believed to affect production adversely.

§Modified Osborne-Mendel salt mixture (after Wesson, L. G., Science 75: 339, 1932). Sodium chloride, 105.00 grams; potassium chloride, 120.00 grams; potassium dihydrogen phosphate, 310.00 grams; tricalcium phosphate, 149.00 grams; calcium carbonate, 210.00 grams; magnesium sulfate (anhyd), 90.00 grams; manganous sulfate (anhyd), 0.20 gram; ferric phosphate, 29.4 grams; potassium aluminum sulfate, 0.09 gram; copper sulfate, 0.39 gram; potassium iodide, 0.05 gram.

TABLE I

NUMBER OF ANIMALS, AVERAGE TOTAL CARIES SCORES, AVERAGE NUMBER OF CARIOUS MOLARS PER ANIMAL, AVERAGE MAXILLARY AND MANDIBULAR SECOND MOLAR SCORES IN TYPICAL ALBINO SERIES

ALBINO FEMALES	FIRST LITTER					SECOND LITTER					THIRD LITTER				
	NUMBER OF WEANLINGS	AVERAGE TOTAL SCORE	AVERAGE CARIOUS MOLARS	SCORES PER 2ND MOLAR		NUMBER OF WEANLINGS	AVERAGE TOTAL SCORE	AVERAGE CARIOUS MOLARS	SCORES PER 2ND MOLAR		NUMBER OF WEANLINGS	AVERAGE TOTAL SCORE	AVERAGE CARIOUS MOLARS	SCORES PER 2ND MOLAR	
				MAX.	MAND.				MAX.	MAND.				MAX.	MAND.
W 32	8	150	12.0	23.8	10.9	8	153	12.0	23.6	15.1	7	72	11.9	19.8	7.1
W 85	7	77	11.6	20.9	6.4	6	89	9.5	21.6	7.3	9	88	11.1	21.4	7.3
W 198	7	89	11.7	22.4	8.5	7	108	11.7	22.8	12.5					
W 200	6	94	10.7	23.1	9.7	4	80	11.0	20	7.1					
W 87	6	59	10.5	18.8	4.4	9	121	11.6	22.9	13.1	7	81	11.6	20.0	5.8
W 584	1	97	12.0	24.0	20.0	4	112	11.5	22.6	10.1					
W 88	8	64	11.5	18.9	5.5	8	79	9.5	22.4	7.7	5	75	10.0	21.9	7.1
W 207	5	88	11.0	20.1	9.9	5	146	11.7	21.5	18.5					
W 334	6	102	9.8	22.3	15.3	8	122	11.9	23.4	19.5	3	0*	0	0	0
W 335	4	136	11.8	23.9	21.1	6	116	11.8	22.5	17.4	6	0*	0	0	0
W 112	7	97	11.1	21.4	10.2	6	128	11.2	23.4	14.6					
W 113	5	55	9.6	15.9	3.9	6	48	10.2	14.4	4.5	5	58	11.6	15.9	4.2
W 116	5	48	10.8	20.3	7.4	6	102	11.8	22.8	12.6	5	90	11.0	20.6	10.0
W 33	6	160	12.0	24.0	15.7	7	114	12.0	23.6	12.5	7	72	10.9	18.9	6.4
W 92	8	100	10.8	22.5	12.4	9	112	11.9	23.9	13.5					
W 30	4	133	12.0	23.6	11.6	5	107	12.0	23.3	9.3					
W 3	5	96	12.0	24.0	18.3	4	134	12.0	20.8	13.7					
W 4	4	148	12.0	23.3	17.1	5	84	12.0	18.4	8.5					
W 8	3	86	11.7	21.0	13.0	6	118	12.0	15.4	7.8	5	126	12.0	22.6	15.7
W 14	6	132	11.4	16.7	6.2	5	62	12.0	20.8	14.6					
W 15	5	145	12.0	21.2	16.0	4	126	12.0	20.6	11.4	3	56	11.3	19.8	9.8
W 73	8	124	11.6	23.1	16.0	8	88	12.0	17.6	5.4	7	37	10.0	9.7	3.6
W 220	5	100	12.0	23.6	13.6	8	148	12.0	24.0	17.6					
W 16	4	130	12.0	24.0	20.8	7	84	11.4	19.9	8.1					
W 18	4	126	11.8	22.3	15.6	6	145	12.0	23.8	14.4					
W 105	7	58	11.3	18.0	5.8	6	53	9.8	18.9	4.5					
W 263	5	68	9.2	20.3	7.8	4	120	12.0	22.4	13.0					
Means	5.5	102.3	11.3	21.6	11.9	6.2	107.4	11.5	21.4	11.6	5.8	75.5	11.1	19.1	7.7

*Caries free litters as a result of continual maintenance on stock diet only. (These values are not included in means.)

crown, and many lesions have not cavitated (Figs. 1, *A* and 4, *D*). To overcome this difficulty, all animals are placed on the ground stock diet mentioned above for a period of 21 days, and at the end of this time they are finally examined and scored (Figs. 1 and 5). A period of one week also seems to be adequate to free the teeth of debris and softened carious material. If animals are to be bred, the males are separated from females upon commencement of the stock diet. Scores are quickly estimated for each tooth on the basis of whole tooth values previously assigned,[3] with approximately six points to a cusp.

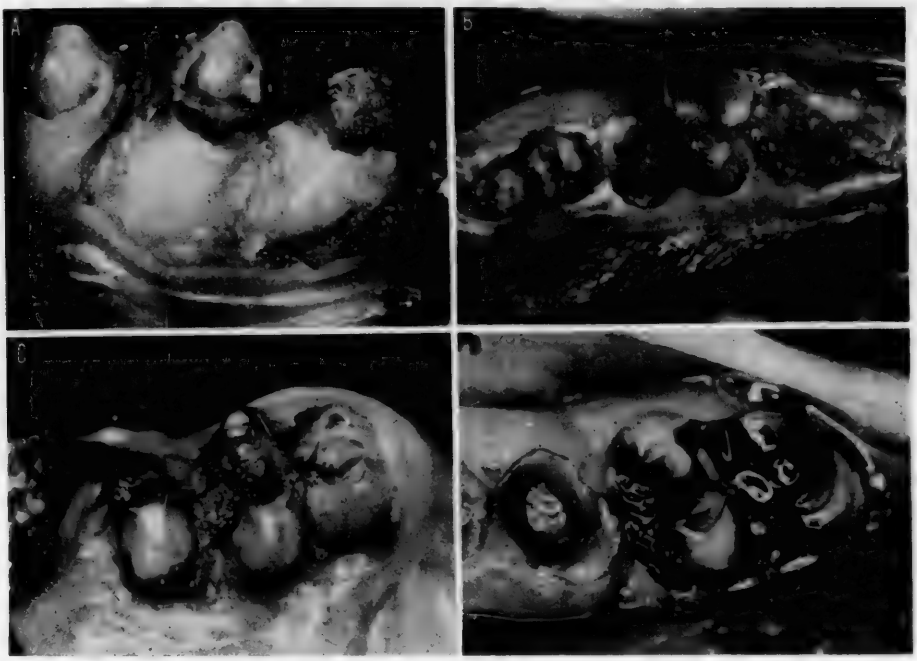

Fig. 1.—Examples of intra-oral photographs taken in live hamsters. *A*, Maxillary second molar covered by plaque which accumulated within a few days after start of the "cariogenic" test diet. *B*, Complete loss of mandibular molars in an animal placed on the experimental diet 35 days and the stock diet an additional 21 days. *C*, Maxillary first molar with coalesced supragingival and subgingival caries. *D*, Mandibular third molar with arrested decay. The second molar has been totally destroyed.

Values can be estimated in round numbers on the basis of the number of cusps missing but those teeth with one or two minute lesions may be scored with a value of less than one, this being the only time fractional values are used.

In regard to scoring, however, under the present experimental conditions, the interpretation of differences between scores is made conservatively. Differences in size of lesions are estimated more closely than may seem necessary to indicate differences of biologic significance. We feel that the most important differences are demonstrated when there is very low to negligible activity in one animal (scores of 0 to 20) and widespread to rampant activity in another (scores of 40 up). The range of activity in a typical sample of "susceptible"

albino animals is illustrated by the graphs in Fig. 2, *A*. The range of male and female scores is illustrated in Fig. 2, *B*. As previously reported,[4, 5] there is a tendency for males to be somewhat more caries active than females; this finding holds even when both sexes are kept together while on the test diet. Cavitation is usually found in ten to twelve molars with a high degree of activity consistently found in second molars (Table I). In some animals, there may be low activity in third molars, seemingly due to slow eruption of this tooth and its resultant short-time exposure to the oral environment (Figs. 1, *D* and 5, *D*). However, it should be re-emphasized that at the present time we are inclined to think of caries activity as much in terms of the animal as a whole as in terms of teeth affected and caries scores.

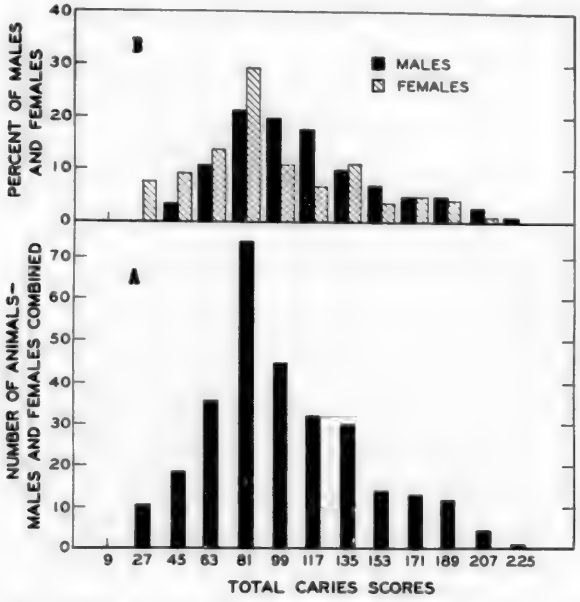

Fig. 2.—*A*, Distribution of total caries scores in a sample of caries active albino hamsters raised and tested under similar laboratory conditions. Scores of inactive animals rarely exceed 20. *B*, The distribution of total caries scores in male and female animals. Activity in males tends to exceed that in females.

The pattern of caries in the albino hamsters does not differ from that in golden animals, but the process is generally more active as evidenced by the fact that the golden animals generally need to be run for 42 days to induce cavitation which approaches that induced in the albinos within 35 days.

In the process of screening hamsters furnished by the animal production station of the N.I.H., a series of 30 albino hamsters were placed on the caries test diet previously described. No caries was observed until the experimental period reached approximately 100 days, at which time lesions had developed in third molars. An assumption, which later proved to be incorrect, was made that these animals might be used to establish a "resistant line." Therefore, several of the most *inactive* males and females were selected for breeding. When

the offspring from these animals were examined after 21 days on the test diet, caries activity was unexpectedly high. Up to seven generations of albino hamsters have now been bred, and none of these animals has shown any loss of activity unless specially treated. In some cases we have bred the same female several times to the same male. While the scores for the litters may vary somewhat as indicated in Table I, the over-all activity of the litters is high. Findings to be published in a subsequent paper strongly suggest that the explanation of the sudden change in "susceptibility" between the first animals tested and the subsequent generations produced from these animals was due to the establishment of a "cariogenic" flora in the alimentary canal of the females. This probably happened during the long exposure to the test diet. No data recorded to date suggest a correlation of caries activity with either gains in weight or size of litter. However, females on the test diet do not gain as well as males and occasionally develop symptoms suggestive of middle ear involvement. Equally active caries has been found in both small and large litters, and there is no evidence that small litters are less active than large ones. (See Table II.)

TABLE II

NUMBER OF ANIMALS PER LITTER COMPARED TO AVERAGE SCORES

	NUMBER IN LITTER							
	2	3	4	5	6	7	8	9
	Average Score Per Litter							
	186	94	80	125	94	72	150	88
	156	131	100	64	98	77	153	121
	72	80	112	75	59	89	79	112
	108	175	136	105	90	108	122	
	156	104	58	88	102	81	100	
		148	108	146	116	97		
		138	191	132	128	114		
		126	130	240	48	72		
		73	68	55	102			
		76	81	58	70			
		137	61	48	99			
		103	104	90	163			
			133	47	126			
				71	98			
					77			
					81			
Mean	136	115	104	96	97	89	121	107

When animals are started on the "cariogenic" test diet, the second molars are almost but not completely erupted. Since the activity in these maxillary teeth is especially high, they will be used to illustrate the initiation and progression of caries now induced. After 2 or 3 days, plaque can be detected on the supragingival surfaces of these teeth, especially in the lingual sulci and occlusal fossae. Plaque also collects rapidly in the surounding gingival crevice and, by 7 days, the accumulation can be as extensive as illustrated in Fig. 1, *A*. The most obvious evidence of caries activity observed in the live animal at this time is a change in color of the tooth. Rather than becoming progressively white, it becomes yellow. At the end of 10 days, gross cavitation can generally be seen

in the live animal and is especially evident at the gingival end of the lingual sulcus of the upper second molar as illustrated by the stripped jaw in Fig. 3, *D*. While enamel changes can be seen in the fossae and sulci of jaws from animals on the diet for 3 days and slight cavitation may occur after 4 to 5 days (Fig. 3, *A*), these early changes are not detectable in living animals. Thus, during

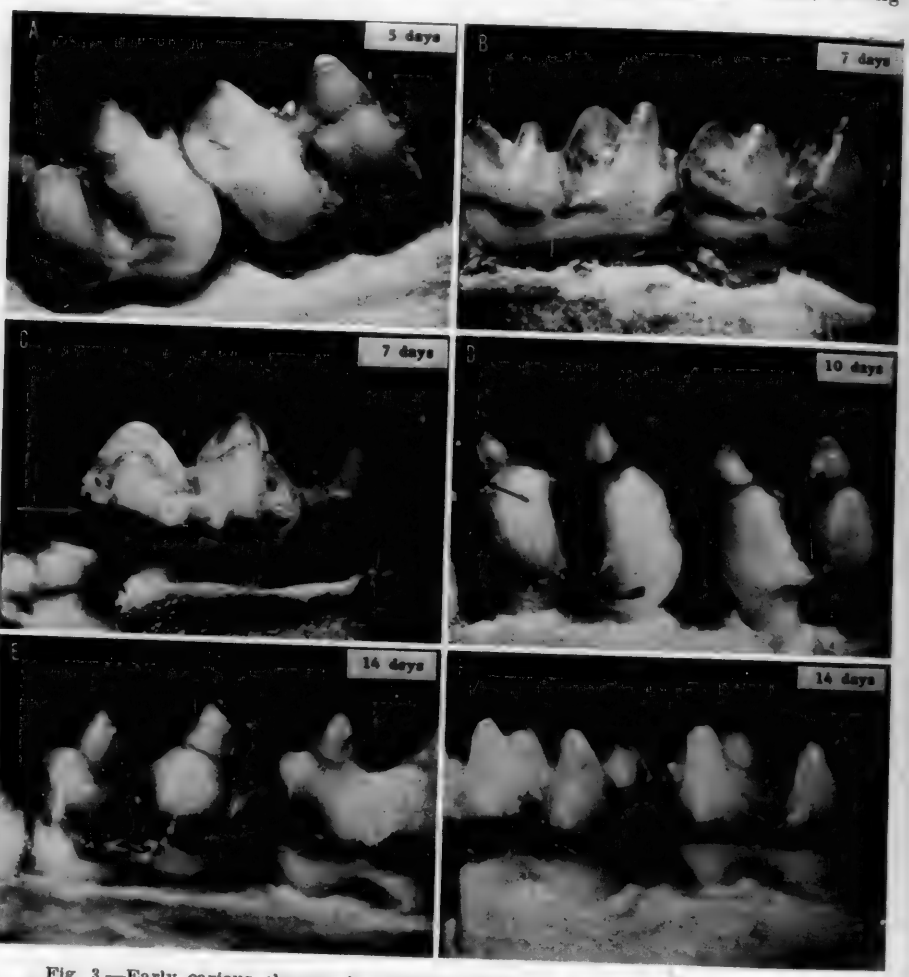

Fig. 3.—Early carious changes in animals exposed to the test diet from 5 to 14 days. *A*, Maxillary first and second molars. *Large arrow* points to area in which slight loss of enamel has already occurred; *small arrows*, to sulcal and lingual surface changes in enamel. *B*, Mandibular lingual surfaces showing location of subgingival enamel caries. *C*, Buccal surface of maxillary second molar with subgingival changes in enamel. *D*, Maxillary second molar with cavitation indicated by *large arrow*. *Small arrow* points to destroyed distobuccal corner of cusp. *E*, Buccal surfaces of mandibular first and second molars with gross cavitation. *F*, Lingual surfaces of molars illustrated in *E*.

the early stages of the disease the extent of caries that can be seen in animals represents only a small fraction of the activity taking place. This is illustrated by the series of photographs taken of teeth in jaws of animals killed at daily intervals over a period of 3 weeks (Figs. 3 and 4).

While the earliest changes in the translucency of enamel have been found in the sulci and fossae, activity in the gingival crevice develops so rapidly that progression from this area is primarily responsible for the massive destruction that occurs (Figs. 4 and 5). In newly erupted teeth, coalescence of lesions is generally so rapid that distinction between gingival crevice and occlusal initiation seems unnecessary and is usually not possible. The variations in the caries patterns between teeth seem to be related to the location of the tooth, its shape, and its age at the time of exposure to the diet. The extensive loss of tooth structure that may occur after 3 weeks on the "cariogenic" diet is illustrated in Fig. 4. At this time, first and second molars may be extensively attacked

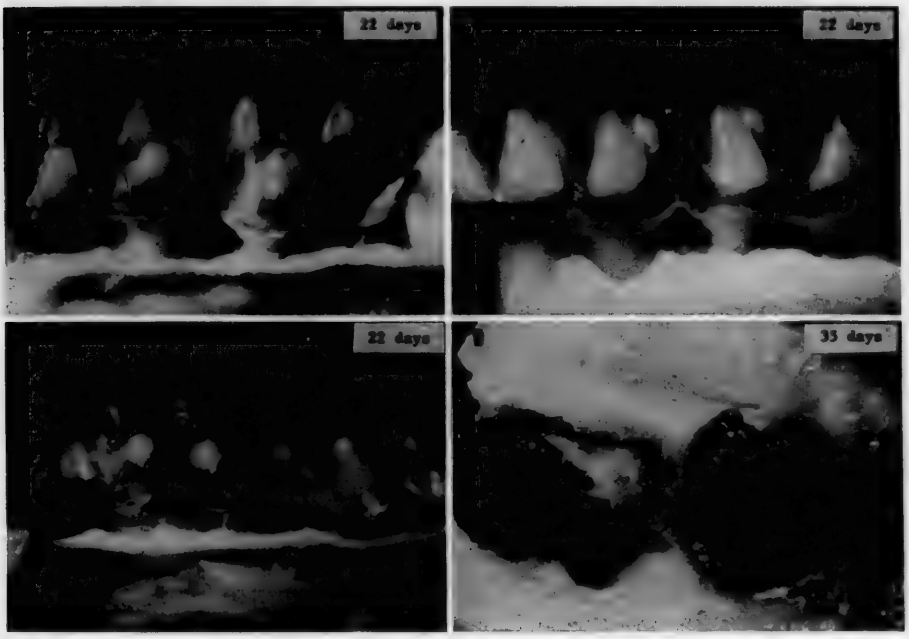

Fig. 4.—*A*, Buccal surface of maxillary first and second molars. Coalesced caries in second molar has destroyed almost half of the tooth. *B*, Extensive cavitation of subgingival origin on lingual surface of mandibular first and second molars. *C*, Rampant caries activity has affected first and second molars of mandible, while newly erupted third molar has not yet been attacked. *D*, Typical appearance of maxillary second and third molars at end of 35-day test period. Extent of activity in the third molar can be determined only after 7 to 21 days on the stock diet, at which time it might resemble the third molar illustrated in either Fig. 5, *C* or 5, *D*.

while the third molars, not long exposed to the oral environment, will not have become involved (Fig. 4, *C*). Occasionally, the penetration of caries is so massive that animals exposed to the test diet for 35 days will lose all the coronal parts of their molar dentition after they have been placed on the stock diet for 21 days (Fig. 1, *D*). However, the more usual patterns of activity are illustrated in Fig. 5.

DISCUSSION

The pattern of caries presently observed differs somewhat from that reported in previous studies[4, 6-9] in which there have been different diets, longer

experimental periods (100 days and over), and generally older animals to start with. Under previous circumstances,[4, 6] there was less massive cavitation, the lesions were more discrete, and sulcal involvement was more evident. Lingual surface lesions on the maxillary first molar were rare and not seen on upper and lower second molars. Proximal lesions were also rare.

It is difficult to compare on a lesion-for-lesion basis the caries activity now seen in hamsters with that in human beings. Many lesions in hamsters appear somewhat analogous to human types because of similarities in location; however, most of the caries activity now induced in hamsters seems to start and progress from the gingival crevice. Present findings suggest that this type of activity

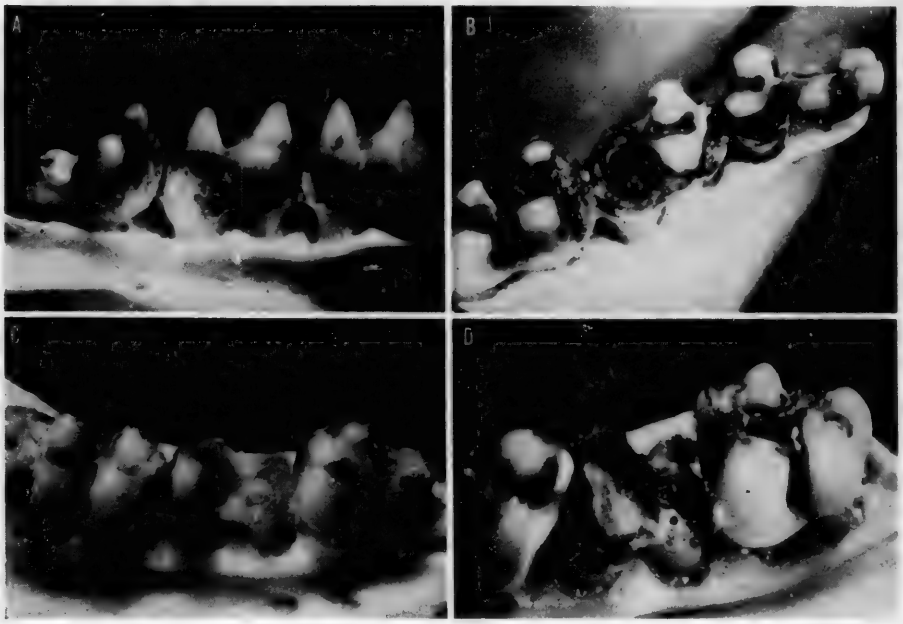

Fig. 5.—Typical examples of caries induced by 35 days on a "cariogenic" diet and arrested by 21 days on the stock diet. *A*, Buccal view of mandible. *B*, Occlusal view of mandible. *C*, Occlusal view of maxilla. *D*, Maxillary second molar has been destroyed while activity in the third molar has been stopped by the change in diet. Initial carious changes in enamel have become deeply pigmented. (See Fig. 1, *D*, also.)

might be more appropriately considered as a crevice type of activity, regardless of the tooth surface involved. For example, despite the difference in location of lesions, it may be that buccolingual cavities in hamsters are more analogous to proximal lesions in human beings than they are to human smooth surface cervical types.

Under the experimental conditions described in this report, it is now possible to determine within a period of 10 or 12 days whether an animal is highly caries active largely on the basis of changes in the upper second molars. Animals selected for breeding can now be assessed after 2 or 3 weeks on the test diet and, if highly active, placed on the stock diet until they reach breeding weight.

The consistency with which caries can be induced in hamsters has been encouraging, and it is now possible to induce cavitation within a few weeks with an excellent degree of predictability. No caries inactive animals have been found in the group of "susceptibles." Reductions in caries activity of major biologic significance are felt to be evident only when activity is fully suppressed or reduced to a negligible range. With the background now available on approximately 2,000 animals, a marked reduction in caries activity becomes immediately apparent and can be detected with certainty in as few as one or two litters and 10 or 12 animals. Since rampant caries can be induced in first and second molars before the eruption of the third molars, it is now possible to manipulate animals so that the pre-eruptive and posteruptive effects of drugs and caries inhibitory agents can be tested.

SUMMARY

A rampant form of dental caries can now be induced in both golden and albino hamsters by feeding a high-carbohydrate low-fat diet. Starting with albino animals about 20 days old, an experimental period of 35 days will generally be adequate for caries activity to cause cavitation in 10 to 12 molar teeth and to destroy many completely.

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SUTURAL EXPANSION BY CONTROLLED MECHANICAL STRESS IN THE RAT

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OSTEOGENESIS is an important phase of sutural growth and can be readily demonstrated in the young animal by a variety of means.[1, 2, 3] Normally, such growth ceases after a given period of time and the suture becomes relatively stable. However, mechanical stress can initiate further sutural activity under certain conditions. Storey, employing helical torsion springs to move incisor teeth, has induced expansion of the premaxillary suture in both rat and rabbit.[4] He has described the lateral movement of the premaxillary bones which followed this procedure and found that the greatest degree of movement occurred near the alveolar crest.

The present investigation was undertaken to develop a method for producing controlled mechanical stress on the premaxillary suture of the rat with the aim of studying the response of the suture to such stress.

METHOD

Forty male albino rats, 2 months of age, housed in wire-bottom cages and fed a stock laboratory diet and water ad libitum, were used in this study. This age ensured an animal young enough to obtain adequate tissue response from mechanical stress and, at the same time, the animal was sufficiently mature that there would be a minimal amount of new bone formation occurring under nonstress conditions, particularly that relating to normal growth.

The premaxillary suture of the rat was employed for the following reasons: (1) This suture remains open throughout most of the life of the rat and was therefore suitable for expansion in a postpuberal animal. (2) The presence of maxillary incisors in close proximity to the suture formed a convenient base for the application of forces. (3) The presence of teeth offered a guide in the uniform orientation of the histologic sections.

A hole, oriented at right angles to the long axis of the tooth, was drilled mesiodistally with a No. 33½ bur through the two maxillary incisors at a constant distance from the gingival crest in all animals. Uniform gum rubber

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wedges of 1/16 inch thickness were inserted between the incisor teeth. A 0.016 inch round steel wire connecting the horizontal holes stabilized the rubber wedge from displacement and minimized excessive tipping of the incisors (Fig. 1, *A*).

The appliance was left in place for 5 days, after which the animals were sacrificed by chloroform inhalation. The heads were decalcified and prepared for sectioning in the routine manner. The maxillary incisor acted as the

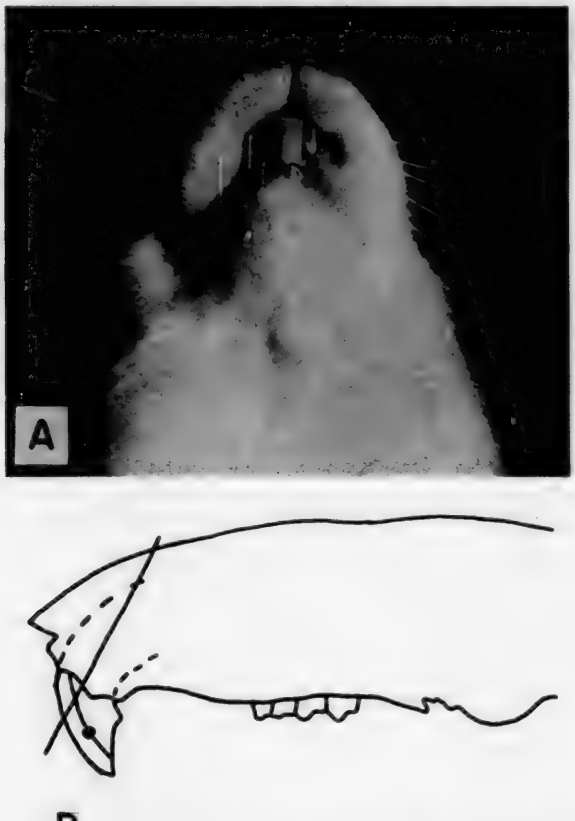

Fig. 1.—*A*, The appliance is shown immediately after insertion and prior to the expansion of the suture. *B*, The oblique line indicates the plane of section used to study the premaxillary suture.

primary guide for orienting the sections. The section was cut perpendicular to the sagittal plane and was determined by two points, one at the alveolar crest and the other 4 mm. apically. This plane passed through the center of the incisor crown at its gingival portion (Fig. 1, *B*).

RESULTS

At the time of sacrifice, there was obvious separation of the maxillary incisor teeth. In a few instances, the rubber wedge had become partly dislodged, but the majority of the animals exhibited an intact appliance.

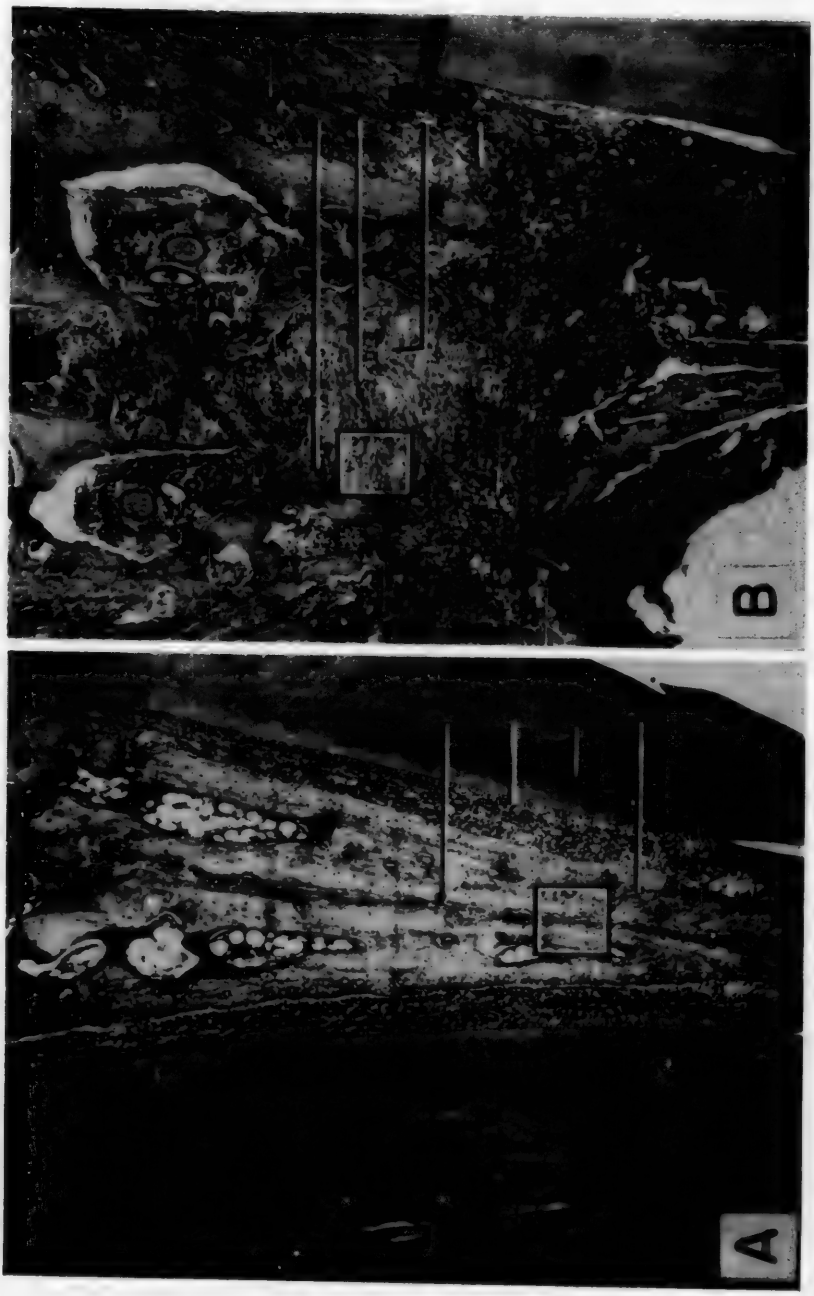

Fig. 2.—The premaxillary suture in the normal (*A*) and the expanded (*B*) state. (*1*) premaxillary suture, (*2*) premaxillary bone, (*3*) periodontal membrane, (*4*) maxillary incisor, and (*5*) osteophytes.

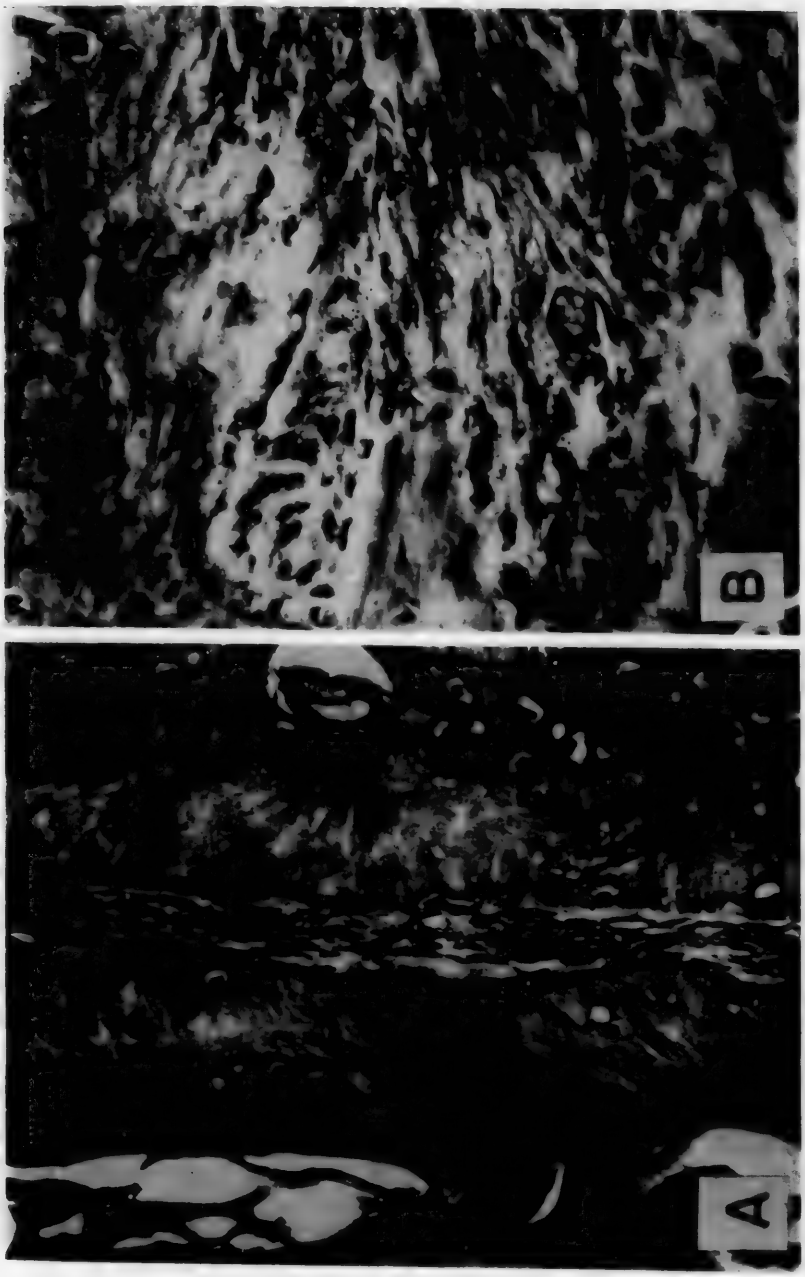

Fig. 3.—Higher power view of areas enclosed by box in Fig. 2.

The normal premaxillary suture in the young rat, in the area under study, measured approximately 20 to 60 μ in thickness (Fig. 2, *A*). The opposing bony plates were separated by thin bands of collagen fibers which, along with interspersed fibrocytes, were oriented parallel to the surfaces of these plates. In some areas, ends of these collagen fibers appeared to be continuous with the very thin band of osteoid which covered the bones at the suture line. Occasional small blood vessels were scattered throughout this connective tissue.

Expansion of the suture by the rubber wedges over a period of 5 days resulted in an opening of this suture to an average width of 377 $\mu \pm 104$ μ (Fig. 2, *B*). There was a considerable amount of new bone formation at the inner surface of the opposing plates of bone and, in most instances, a remarkable osteophyte response. The general orientation of the connective tissue fibers changed from a parallel (Fig. 3, *A*) to a perpendicular direction (Fig. 3, *B*), with fibers extending across the suture between the two surfaces of bone. There was also an increase in the number of fibroblasts present, as well as increased vascularity in the sutural connective tissue.

The osteophytes appeared first as a condensation of the collagen, with osteoid in the form of small bars extending out into the sutural connective tissue. Osteoid and ultimately bone was formed between these bars on the surfaces of the opposing plates. Sometimes the osteophytes were of such length that they interdigitated across the suture line. These bony projections were always bordered by active osteoblasts.

The rubber wedge in some instances had been forced against the tissue under a pressure that resulted in an intense inflammatory reaction in the crest of the interdental tissues. This reaction occasionally extended a short distance into the medial periodontal membrane of the incisors and commonly resulted in necrosis and sequestrum formation at the tip of the alveolar bone approximating the premaxillary suture.

The lateral pressure exerted by the rubber wedge was reflected in changes in the periodontal membrane and alveolar bone similar to those described in experimental studies of controlled tooth movement. The periodontal membrane at the coronal portion of the alveolus was compressed on the pressure side and showed hyalinization with loss of viable cells. The adjacent bone showed osteoclastic activity. On the tension side, the periodontal membrane was wider than usual and the alveolar bone exhibited spurs of new bone formation, the appearance of which was generally correlated with the formation of new bone along the suture line in any given animal. Thus, some animals exhibited a relatively small amount of new bone in the suture and in the periodontal membrane, whereas other animals formed considerable new bone in both locations. Such a finding might suggest a normal range of variability in the osteogenic potential of untreated, control animals.

DISCUSSION

The use of the rubber wedge for sutural expansion offers a simple, reproducible technic with neglible appliance breakage and low animal mortality.

Even though individual variation in the tissue response is found, the relative uniformity of the sections when properly oriented suggests the possible application of this method to studies relating endocrine metabolism, vitamin metabolism, mineral metabolism, and other extrinsic and intrinsic factors with osteogenesis. This approach might have certain advantages over investigations utilizing animals with growing sutures since osteogenesis is more pronounced in the artificially expanded suture and differences might be made more evident. Furthermore, in order to gain adequate tissue response, it would no longer be necessary to depend on immature animals. This is particularly desirable in experiments involving the endocrine system.

The rapidity of response of the suture to the rubber wedge is quite remarkable and is indicative of the high potential for connective tissue and bone activity at a site of relative quiescence.

Weinmann and Sicher have described a division of sutural connective tissue into three layers: a central layer consisting of a "feltwork" of connective tissue fibers rich in cells and a layer on either side, bordering the bone, made of denser connective tissue and fewer cells. In the expanded suture, the proliferating connective tissue cells were diffusely dispersed throughout the entire width of the suture and no tendency for stratification was evident. This difference may be explained by the nature of the stimulus and the rapidity of response to it in the artificially expanded suture.

An interesting variation in response of control animals was observed in this study, namely, the amount of new bone formed in the expanded suture. Some animals exhibited long osteophytes projecting into the suture line from the plates of bone while other animals showed no such bony formations, even though the actual width of sutural expansion was approximately the same in each instance. A similar situation was noted in these animals with respect to new bone formation in the periodontal membrane on that side of the tooth under tension. Some animals demonstrated considerable new bone, others very little. This would imply that even in an inbred strain of animal there are certain unrecognized factors which produce distinct variations in the osteogenic potential and that such factors must be recognized and considered when drawing concrete conclusions.

SUMMARY AND CONCLUSIONS

A simple and reliable method for inducing premaxillary suture expansion in the rat has been presented. This technic has utilized the pressure released from deformed gum rubber wedges which were stabilized between the maxillary incisor teeth.

There was histologic evidence of reorientation of connective tissue fibers between the opposing plates of bone with the formation of osteophytes, sometimes interdigitating, projecting into the expanded suture line. These findings indicate that a quiescent suture can be activated by the application of an external mechanical stress.

This method could be readily adapted to investigations relating endocrine metabolism, vitamin metabolism, mineral metabolism, and other extrinsic and intrinsic factors with osteogenesis.

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DECOMPOSITION OF GYPSUM INVESTMENT IN THE PRESENCE OF CARBON

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I. INTRODUCTION

IT HAS been observed for years that overheating gypsum content investments results in the formation of corrosive sulfur compounds. Difficulty in pickling a gold casting is an indication of the formation of an insoluble sulfide on the surface of the casting. The effects of sulfur contamination are particularly marked when a gold casting is embedded in investment with the object of casting to it. This casting technic is used to add a clasp, bar, or backing to a denture, or casting to a gold coping. Besides making the embedded casting structurally weak, a sulfide surface film will prevent a good joint between two castings. Sulfur contamination will also result from overheating soldering investment with a torch.

The decomposition of calcium sulfate in investments has been mentioned in many references on dental gold casting procedures. Although the process is a chemical reaction, equations and equilibrium data are lacking in the literature. There is also uncertainty about the conditions under which the reaction will take place. It is, therefore, the purpose of this paper to show the reaction mechanism and describe the conditions under which the reaction takes place.

Souder and Paffenbarger[1] stated that gypsum investments will give off sulfur gases if heated much above 700° C. Shell[2] and Brumfield[3] related sulfide contamination to the decomposition of calcium sulfate when heated above 700° C.

According to Brumfield[3] the gypsum $(CaSO_4)_2H_2O$ content of dental gold casting investments varies from 25 to 50 per cent of the total weight. It may be in the form of plaster or hydrocal. The other major constituent of these investments is silica (SiO_2). Some other ingredients present in small amounts are: boric acid, graphite, sodium chloride.

II. PROCEDURE

A. *Qualitative Tests.*—

In order to determine the temperature at which calcium sulfate will decompose under various conditions, the horizontal tube furnace shown in Fig. 1 was used. The samples tested were: (1) C. P. calcium sulfate; (2) C. P. calcium

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sulfate + 2 per cent powdered graphite; (3) an A.D.A. certified cristobalite inlay investment which did not contain carbon; and (4) same investment as in (3) + 2 per cent added graphite.

Fresh charges were heat soaked at 100° intervals for one hour and then dried air was passed over the charge and collected in a gas absorption bulb. The temperature was regulated by means of a photoelectric temperature controller to ± 5° C. Solutions of potassium permanganate and barium chloride were used to test for the presence of sulfur dioxide and sulfur trioxide, respectively.

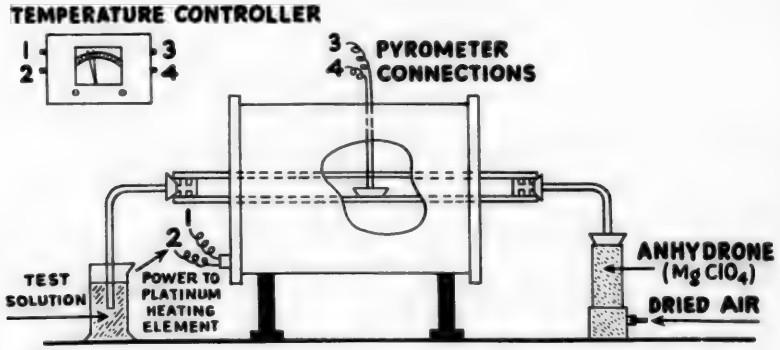

Fig. 1.—Furnace used to determine decomposition temperature of gypsum.

B. *Quantitative Analysis.*—

The percentage reduction of calcium sulfate by carbon and carbon monoxide was determined by analyzing the residue of the above tests for calcium sulfide. The analysis was performed according to A.S.T.M. No. E30-47, Section 52, for the determination of metallic sulfides. The sample was heated with hydrochloric acid and the hydrogen sulfide evolved was dissolved and titrated iodiometrically. This experiment was repeated with carbon monoxide and the same analysis made.

C. *Metallographic Experiment.*—

The following alloy was used to determine the effect of investment decomposition on microstructure: Au 65 parts by weight, Pd 5 parts by weight, Cu 9 parts by weight, Ag 20 parts by weight, and Zn 1 part by weight.

This alloy is referred to as Alloy I in the following text. One inch long, ten gauge rods were cast with Alloy I. The following mixtures were placed in individual porcelain boats and one rod buried in each: (1) C. P. calcium sulfate; (2) C. P. calcium sulfate + 2 per cent graphite; (3) A.D.A. certified cristobalite inlay investment which did not contain graphite; and (4) same investment as in (3) + 2 per cent graphite.

The boats were then placed in an electric furnace and held at 760° C. for one hour. A control was furnished by heating a rod alone at 760° C. for one hour.

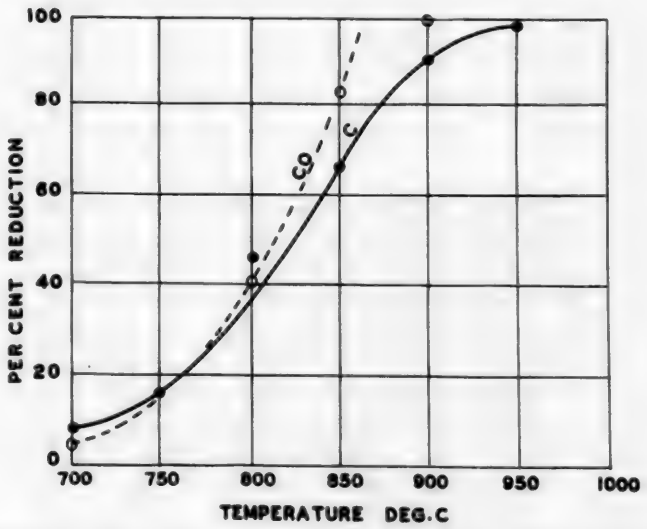

Fig. 2.—Experimental data showing per cent reduction of calcium sulfate by carbon and carbon monoxide at various temperatures.

TABLE I

TEMPERATURE (°C)	K Mn O_4 DISCOLORATION	Ba Cl_2 PRECIPITATION
	1. *C. P. Calcium Sulfate*	
500	None	None
600	None	None
700	None	None
800	None	None
900	None	None
1,000	None	None
1,100	None	None
1,200	Slight	Light
1,300	Slight	Light
	2. *C. P. Calcium Sulfate + 2 Per Cent Graphite*	
500	None	None
600	None	None
700	Slight	None
800	Rapid	Light
900	Immediate	Heavy
	3. *Nongraphitic Inlay Investment*	
500	None	None
600	None	None
700	None	None
800	None	None
900	None	None
1,000	Slight	None
1,100	Rapid	Light
	4. *Nongraphitic Inlay Investment + 2 Per Cent Graphite*	
500	None	None
600	None	None
700	Slight	Light
800	Rapid	Light
900	Immediate	Heavy

III. RESULTS

A. *Qualitative Analysis.*—The qualitative analyses are given in Table I.

B. *Quantitative Analysis.*—

A graph showing the percentage reduction of calcium sulfate by carbon and carbon monoxide is shown in Fig. 2.

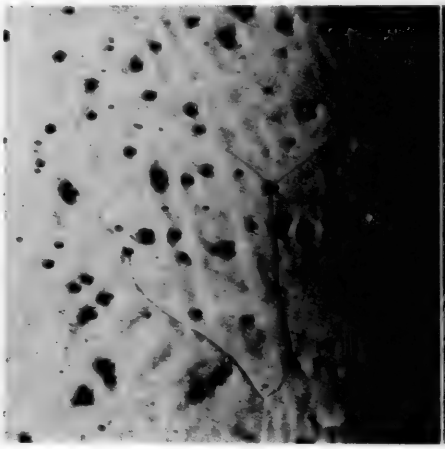

Fig. 3.

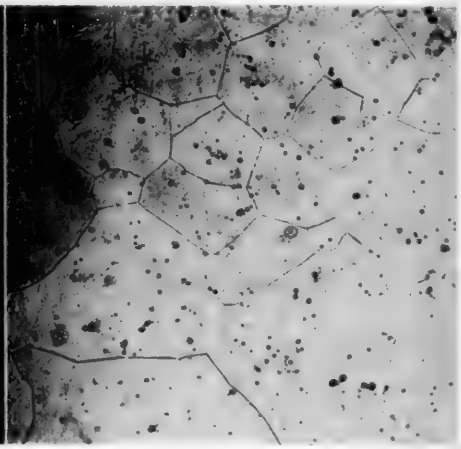

Fig. 4.

Fig. 3.—Control microstructure of cast Alloy I rod heated alone for one hour at 760° C. Cut along axis. Etched with KCN + $(NH_4)_2S_2O_8$. (Orig. mag. ×50.)

Fig. 4.—Microstructure of cast Alloy I rod embedded in C. P. calcium sulfate for one hour at 760° C. Cut along axis. Etched with KCN + $(NH_4)_2S_2O_8$. (Orig. mag. ×50.)

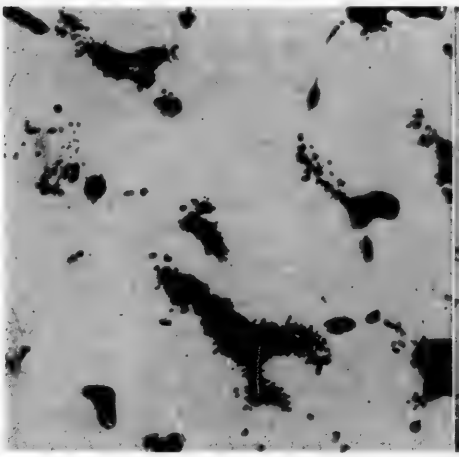

Fig. 5.

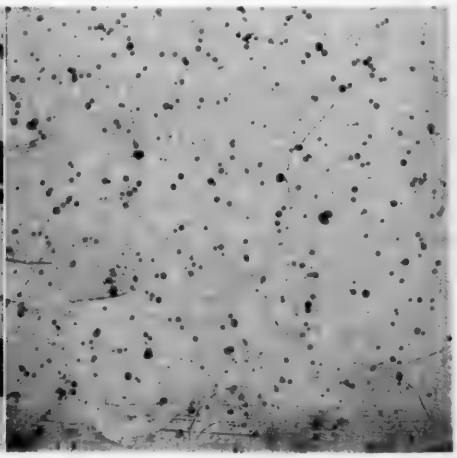

Fig. 6.

Fig. 5.—Microstructure of cast Alloy I rod embedded in mixture of C. P. calcium sulfate and 2 per cent carbon for one hour at 760° C. Cut along axis. Etched with KCN + $(NH_4)_2S_2O_8$. (Orig. mag. ×50.)

Fig. 6.—Microstructure of cast Alloy I rod embedded in a cristobalite inlay investment for one hour at 760° C. Cut along axis. Etched with KCN + $(NH_4)_2S_2O_8$. (Orig. mag. ×50.)

C. *Metallographic Results.*—

The control (Fig. 3) shows a fine distribution of pinhole pits probably due to the etching out of a minor constituent resulting from the 760° C. heating.

Figs. 4 and 6 show the results of heating Alloy I in contact with C. P. calcium sulfate and a nongraphitic inlay investment, respectively. There is no indication of sulfur contamination in either case.

Figs. 5 and 7 show a heavy etching attack of regions of copper sulfide, mostly at the grain boundaries for the case of calcium sulfate + 2 per cent graphite and the case of the cristobalite inlay investment + 2 per cent graphite, respectively.

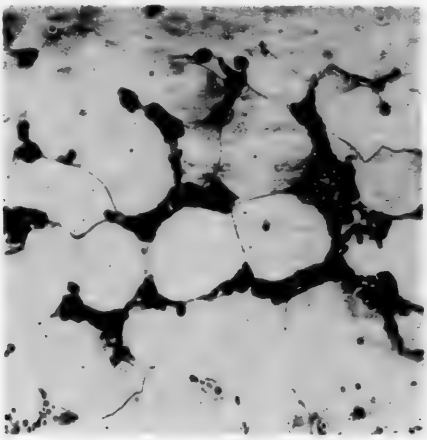

Fig. 7.—Microstructure of cast Alloy I rod embedded in a mixture of a cristobalite inlay investment and 2 per cent carbon for one hour at 760° C. Cut along axis. Etched with KCN + $(NH_4)_2S_2O_8$. (Orig. mag. ×50.)

IV. DISCUSSION

Thermodynamics is being widely used in chemistry as a tool in predicting equilibria in chemical reactions. The free energy change of a reaction is the criterion in judging whether a reaction will take place at a particular temperature and pressure. The free energy change and temperature are related to the equilibrium constant of a reaction by the following:

$$\Delta F^\circ = -RT \ln K_p.$$

ΔF° is the free energy change of the reaction in calories. R is the universal gas constant in calories per degrees per mole. T is the temperature in degrees Kelvin. K_p is the equilibrium constant for the reaction using partial pressures in atmospheres.

A reaction can take place spontaneously only if it is accompanied by a decrease in free energy. The free energy change shows a decrease by a negative sign. A positive free energy change indicates that the reaction will not take place at the temperature indicated.

This criterion may be used to show why calcium sulfate will not decompose without reducing agents at 700° C. The equation for the decomposition of calcium sulfate by itself and the accompanying free energy change is:

$$CaSO_4 = CaO + SO_3 \quad (1) \quad \Delta F° = +50{,}000 \text{ calories at } 700° \text{ C.}$$

This positive free energy change shows that the reaction will not take place at the temperature stated in the literature for the release of sulfur compounds.

Investigation of the chemistry of sulfates shows that they are susceptible to reduction by carbon or carbon monoxide. This is well known in the field of quantitative analysis. In the determination of sulfur by the barium sulfate method, care is cautioned in the ignition of barium sulfate to prevent reduction by the carbon of the filter paper. The reaction is given as:

$$BaSO_4 + 4C = BaS + 4\ CO \quad (2).$$

The analogous reaction with calcium sulfate is:

$$CaSO_4 + 4C = CaS + 4\ CO \quad \Delta F° = -47{,}000 \text{ at } 700° \text{ C.}$$

The free energy change values show the reduction of calcium sulfate to be the most probable reaction responsible for the formation of sulfur gases at 700° C. The calcium sulfide will react with any copper oxide in the alloy to form copper sulfide.

$$CaS + Cu_2O \text{ (in Au-Cu)} = CaO + Cu_2S \quad \Delta F° = -21{,}270 \text{ calories at } 700° \text{ C.}$$

Fig. 8.—Cross section of a mold showing carbon residue remaining after burnout in a reducing atmosphere.

If calcium sulfide has not been formed during wax elimination, it is unlikely that it will form during the casting of gold alloys with casting temperatures below 1,150° C. There are two reasons for this:

1. The thermal gradient between the molten alloy and the mold wall is great enough to prevent the mold temperature from rising to that of the alloy temperature.

2. The rise in mold temperature during casting is of short duration. Insufficient time above 700° C. is available for the reaction to reach equilibrium.

The most effective method of avoiding the reduction of calcium sulfate is to keep the temperature below 700° C. This will prevent reduction even if carbon is present. If a 700° C. mold temperature is desired, contact with any reducing agents must be avoided. The carbon residue left by a wax

pattern is a source of sulfur contamination if still present at 700° C. Fig. 8 shows the cross section of a mold burned out at 700° C. in a reducing atmosphere. Investments containing graphite should not be heated above 700° C. during wax elimination. Contact between the blow torch flame and investment should be minimized during brazing (soldering) operations.

V. SUMMARY AND CONCLUSIONS

An experimental study of the decomposition of gypsum investments showed the following: (1) calcium sulfate by itself will not decompose until heated to 1,200° C., and (2) the reduction of calcium sulfate by carbon takes place rapidly above 700° C. releasing calcium sulfide and sulfur dioxide.

$$CaSO_4 + 4C = CaS + 4CO$$

$$3\ CaSO_4 + CaS = 4CaO + 4SO_2.$$

This reaction will take place if gypsum investments are heated above 700° C. in the presence of carbon or carbon monoxide. The products of this reaction contaminate gold castings and make them brittle.

The authors are indebted to P. H. Herasymenko for his help in checking the calculations.

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URINARY FLUORINE LEVELS IN WOMEN DURING PREGNANCY AND AFTER DELIVERY

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EARLIER studies have demonstrated that the fluorine level in the urine of normal adults, drinking naturally or artificially fluoridated water, is very close to the level of fluorine in the drinking water.[1-6] In children 5 to 14 years old, who had previously drunk fluorine-free water, it was found that the level of fluorine in the urine rose to 1 ppm 3 to 5 years after they had started to drink water artificially fluoridated to 1 ppm.[6] Longwell[7] established that the fluorine content of the urine from 36 children 5 to 12 years old, living in the London area was 0.16 to 0.35 ppm, the older children having the higher values. In Jerusalem which is supplied with drinking water containing 0.5 to 0.6 ppm fluorine[8, 9] it was found that the mean urinary fluorine content in children, 1 to 3 years old, was 0.14 ppm, and of children 4 to 6 years old, 0.27 ppm.[10] These were significantly lower than the value in adults, 0.52 ppm, which is approximately the same as the fluorine content of the drinking water. The data of these studies suggest strongly that storage of fluorine in the body takes place during life, predominantly during childhood, and that the proportion of ingested fluorine which is retained decreases as the child grows older.

In the present investigation the urinary fluorine level of women at various stages of pregnancy was estimated in order to study the influence of the developing fetus on the fluorine metabolism of the mother.

MATERIALS AND METHODS

Samples of urine voided between 9 A.M. and noon were obtained from 117 healthy pregnant women residing in Jerusalem. All these pregnancies were normal. The collection of 24-hour urine specimens from persons not under hospital supervision is practically impossible. According to observations made by Zipkin, Likins, McClure, and Steere,[6] no appreciable variations in the urinary fluoride content occur with the time of day the specimen is passed; preliminary determinations of our own in several pregnant and nonpregnant women agreed with their findings (Table I).

Furthermore, in an earlier study, we examined the fluorine concentrations in the urine of the same person on different days over a period of about one month; it was found that the deviations from the mean value lay within the

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TABLE I

FLUORINE EXCRETION (PPM) IN URINE SAMPLES TAKEN FROM INDIVIDUAL PREGNANT AND NONPREGNANT WOMEN DURING 24 HOURS*

PREGNANT WOMEN	MONTH OF PREGNANCY	TIME SAMPLE TAKEN						MEAN F VALUE (PPM)
		A.M.			P.M.			
		6.30	8.30	11.30	2.30	5.30	9.30	
Case I	3			0.60	0.60		0.60	0.60
Case II	8	0.30	0.45	0.30	0.30	0.35	0.25	0.33
Nonpregnant women								
Case III		0.60	0.50	0.60	0.50	0.65	0.50	0.56
Case IV		0.60			0.60	0.60		0.60

*The experimental error of the analytical method used is ± 0.10 ppm F.

limits of the experimental error (±0.1 ppm) of the analytical method used.[10] Consequently, we considered it justifiable to take one urine sample per month from each woman, the first, during the fourth month of pregnancy at the first visit to the Mother and Infant Welfare Station (Table II, Group A). Urine samples from some of the cases were examined for fluorine after delivery up to a period of 3 months.

TABLE II

MEAN FLUORINE CONCENTRATION IN URINES OF PREGNANT WOMEN AT MONTHLY INTERVALS

GROUP	MONTH OF PREGNANCY*	NO. OF CASES†	VALUE OF FLUORINE (PPM)			MONTHS COMPARED	SIGNIFICANCE OF DIFFERENCE ($P < 0.01$)	p
			MEAN	MAX.	MIN.			
A	4	117	0.53	0.80	0.20			
	5	80	0.43	0.70	0.10	4:5	+	0.0001
	6	91	0.34	0.65	0.10	5:6	+	0.0001
	7	89	0.28	0.65	0.0	6:7	+	0.0001
	8	81	0.22	0.65	0.0	7:8	+	0.0001
	9	88	0.29	0.70	0.0	8:9	+	0.0001
	Month after delivery							
	1	55	0.39	0.80	0.10	9:1	+	0.0001
	2 + 3	64	0.49	0.80	0.30	1:(2 + 3)	+	0.0001
B	4	45	0.50	0.80	0.30	4A:4B	-	0.16
C	8	32	0.23	0.35	0.05	8A:8C	-	0.50

*It was not possible to make a more precise estimation of the duration of pregnancy in weeks.

†The number varies from month to month because not all the women attended regularly.

Urinary fluorine was also examined in two additional groups: one group of 45 women in the fourth month (Group B), and the other of 32 women in the eighth month of pregnancy (Group C).

All samples were analyzed for fluorine according to standard procedure as previously described by one of us.[10]

RESULTS

Group A.—The mean urinary fluorine concentration of the women in the fourth month of pregnancy was 0.53 ppm, that is, almost equal to the mean fluorine concentration of the urine in adult males and nonpregnant adult

females, 0.52 ppm, drinking water with 0.5 to 0.6 ppm F. In the later months of pregnancy, the value decreased gradually, reaching its minimum, 0.22 ppm, in the eighth month. In the ninth month, the urinary fluorine level started to rise, reaching 0.29 ppm. After delivery it rose further, the mean value in the following month being 0.39 ppm and during the next 2 months 0.49 ppm, which approaches the mean fluorine urinary concentration of adult males and non-pregnant adult females drinking naturally fluoridated water.[10]

Table III shows the wide individual variation in the distribution of the urinary fluorine levels at each month of pregnancy and after delivery.

Groups B and C.—The results were not significantly different from those in Group A at the equivalent month of pregnancy (Table II).

TABLE III

DISTRIBUTION OF FLUORINE CONCENTRATION IN ALL URINE SAMPLES TAKEN: DURING PREGNANCY AND AFTER DELIVERY

MONTH OF PREGNANCY	NUMBER OF CASES	MEAN F VALUE (PPM)	DISTRIBUTION NUMBER OF F CONCENTRATION (PPM)								
			0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
4	117	0.53			1	5	37	29	30	14	1
5	80	0.43		1	1	22	32	15	6	3	
6	91	0.34		9	16	38	20	7	1		
7	89	0.28	1	11	35	26	14	0	2		
8	81	0.22	4	20	36	20	0	0	1		
9	88	0.29	5	14	24	17	12	8	5	1	
Month after delivery											
1	55	0.39		2	7	14	15	8	6	2	1
2 + 3	64	0.49				2	22	22	14	3	1

DISCUSSION

The lowered urinary excretion of fluorine in pregnant women indicates that retention of the ingested fluorine is taking place.[2] Retained fluorine probably accumulates in placental tissue, since Gardner, Smith, Hodge, Overton, and Feltman[11] and Feltman and Kosel[12] have found that a positive correlation exists between fluorine ingestion and the fluorine concentration of the placenta. However, the placenta does not act as barrier to the transference of trace quantities of fluorine from mother to fetus after calcification has begun and thus it is likely that fluorine is also stored in the fetus.[11-15] It is of interest to note that the decrease in urinary fluorine, which starts at about the fifth month of pregnancy, coincides with the commencing mineralization of the deciduous teeth.[16, 17, 18] The rising urinary excretion beginning in the ninth month may indicate that fluorine storage in the fetus is approaching completion.[19] The urinary fluorine level did not return to the usual adult mean value immediately after delivery[18]; a further investigation of fluorine metabolism of pregnant and lactating women is in progress to elucidate the factors involved.

SUMMARY

Seven hundred fifty-one urine samples obtained from 196 women during pregnancy and after delivery were analyzed for fluorine. All these women used

the same water supply. The urinary fluorine levels decreased progressively up to the eighth month of pregnancy, indicating a retention of fluorine during this period. After pregnancy the urinary fluorine level did not immediately return to the usual adult value.

The authors are indebted to Dr. G. Kallner from the Central Bureau of Statistics for the statistical evaluation of the results.

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HISTOLOGIC OBSERVATIONS ON THE MORPHOLOGY OF THE BLOOD VESSELS OF CANINE AND HUMAN TOOTH PULP

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INTRODUCTION

THE morphology and distribution of blood vessels within the tooth pulp have been the subject of relatively few studies in recent years.[1, 2, 3] These more recent studies, like the older ones,[4, 5, 6] are primarily concerned with the degree of vascularity and the gross morphologic picture as determined by various modifications of the familiar method of injecting ink, stains, and colored plastics into the vessels; however, these previous studies have all failed to point out one interesting aspect of the morphology of the blood vessels which the authors have repeatedly observed while examining both canine and human tooth pulps. The diameters of both the arteries and veins, which are present in the pulp, were observed to be greater in the mid-region of the pulp and smaller toward the apical and coronal terminals. This condition appears to be parallel to that known to exist in the blood vessels of the wall of the alimentary tract, where the veins and arteries are greater in diameter in the area immediately under the surface mucosa. The following data represent a detailed histologic study of this unusual condition relative to the diameters of the blood vessels.

MATERIALS AND METHODS

Twenty-one noncarious teeth, extracted from dogs approximately 3 years old, were fixed in neutral formalin (commercial formalin neutralized with sodium hydroxide and repeatedly checked by means of a Beckman pH meter). The duration of fixation ranged from 24 to 72 hours without resulting in any visible histologic differences. The pulps were then removed by cracking the enamel and dentin in much the same manner as that described by Provenza,[7] and immediately cut serially in both longitudinal and cross sections by means of the standard histologic technics of dehydrating in an ethyl alcohol series and imbedding in paraffin. The cross sections were cut at 10 μ in thickness and the longitudinal sections at 8 μ in thickness.

Twenty noncarious human deciduous and 25 adult teeth were fixed in neutral formalin while others were fixed in 80 per cent ethyl alcohol; all of

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these were sectioned serially in a like manner as that just described. Subsequent comparisons failed to reveal any histologic differences in the pulps fixed by the two methods.

The sectioned tissues were stained with (1) hematoxylin and eosin; (2) Mallory's triple connective tissue stain; (3) benzidine for occult blood as described by Washburn,[8] and (4) Gomori's trichrome stain[9] with a slight modification (Harris' alum hematoxylin was used instead of Weigert's as the nuclear stain).

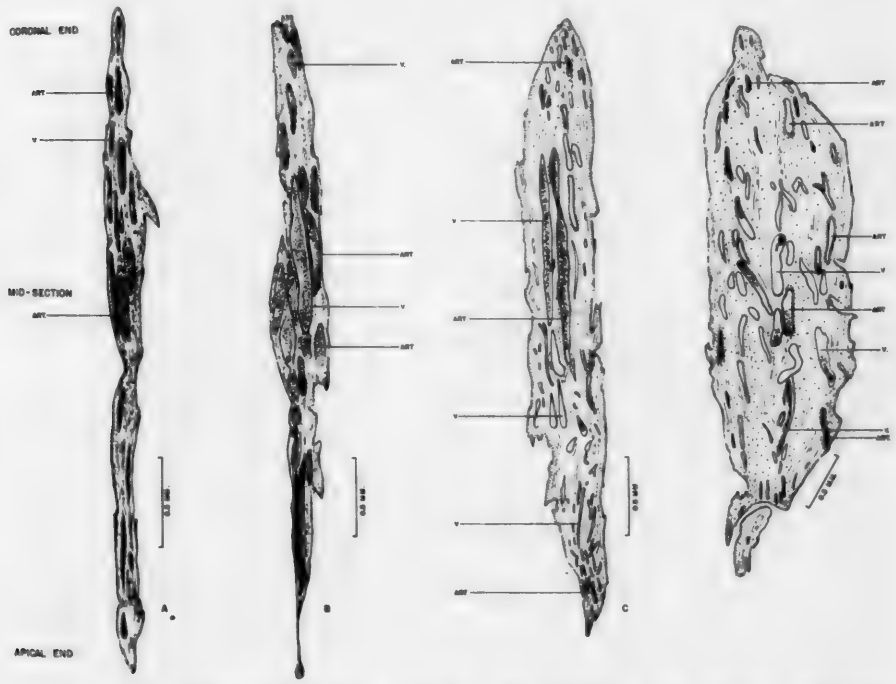

Fig. 1.—*A*, Longitudinal section of pulp of upper incisor of dog showing the greater diameters of the blood vessels in the mid-section.

B, Longitudinal section of pulp of lower incisor of dog showing the greater diameters of the blood vessels in the mid-section.

C, Sagittal section of human bicuspid (pulp) showing the greater diameters of the blood vessels in the mid-section.

D, Mediosagittal section of human bicuspid (pulp) showing the greater diameters of the blood vessels in the mid-section. *V*—Vein; *ART*—Artery.

The benzidine stain was used with the idea that the occult blood in the vessels would take on the characteristic blue-black color, thus permitting immediate identification of the vessels. This attempt was quite satisfactory. All measurements were made from medial sections with the aid of a calibrated eye-piece micrometer. Only the medial sections were used since the dimensions from such are the only ones which are reliable, inasmuch as the blood vessels are tridimensional.

HISTOLOGIC OBSERVATIONS

In dog pulps from the upper and lower incisors, the arteries entering through the apical foramen measure 0.011 to 0.037 mm. in diameter (average 0.025 mm.), while the veins in the same area measure 0.014 to 0.042 mm. in diameter (average 0.0275 mm.). These estimates are based on the dimensions of twenty-one vessels of each type. A certain degree of anastomosis occurs shortly after the artery(ies) enter the apical foramen. The branches of the arteries become larger in diameter; those observed in the mid-zone of the pulp measuring 0.042 to 0.075 mm. in diameter (average 0.059 mm.). The veins found in the same region measure 0.053 to 0.112 mm. in diameter (average 0.071 mm.). These measurements are based on the diameters of forty-one vessels of each type.

Further anastomoses occur as the arteries extend toward the coronal end; however, the blood vessels become smaller in diameter. The arteries found near the coronal end measure 0.03 to 0.045 mm. in diameter (average 0.0385 mm.), while the veins measure 0.0375 to 0.06 mm. in diameter (average 0.0407 mm.). These measurements are based on the diameters of sixteen blood vessels of each type.

All the human teeth used in this study were bicuspids and are herein separated into two categories, deciduous and adult. Table I includes the measurements of the diameters of the blood vessels in both categories.

TABLE I
COMPARATIVE MEASUREMENTS OF THE DIAMETERS OF VEINS AND ARTERIES IN THE HUMAN TOOTH PULP
(ALL MEASUREMENTS GIVEN IN MILLIMETERS)

	ARTERIES			VEINS		
CATEGORY OF TEETH	RANGE	AVERAGE	NO. OF VESSELS MEASURED	RANGE	AVERAGE	NO. OF VESSELS MEASURED
Deciduous Teeth						
Apical region	0.007-0.030	0.0169	15	0.007-0.0375	0.0256	40
Mid-region	0.045-0.075	0.0605	13	0.030-0.060	0.0384	10
Coronal region	0.015-0.060	0.0229	17	0.015-0.045	0.0265	33
Adult Teeth						
Apical region	0.015-0.030	0.0208	10	0.015-0.060	0.030	10
Mid-region	0.045-0.075	0.0609	10	0.030-0.120	0.0712	43
Coronal region	0.0225-0.0525	0.0325	30	0.015-0.0375	0.021	32

The arteries can be easily separated from the veins by their histologic constitution where hematoxylin and eosin, Mallory's, and Gomori's trichrome stained sections are studied. The walls of the arteries are thicker and their lumina are smaller. From the data presented in the chart of comparative measurements, it can be readily appreciated that, in each of the three designated regions, the veins are usually larger in diameter than are the arteries; this

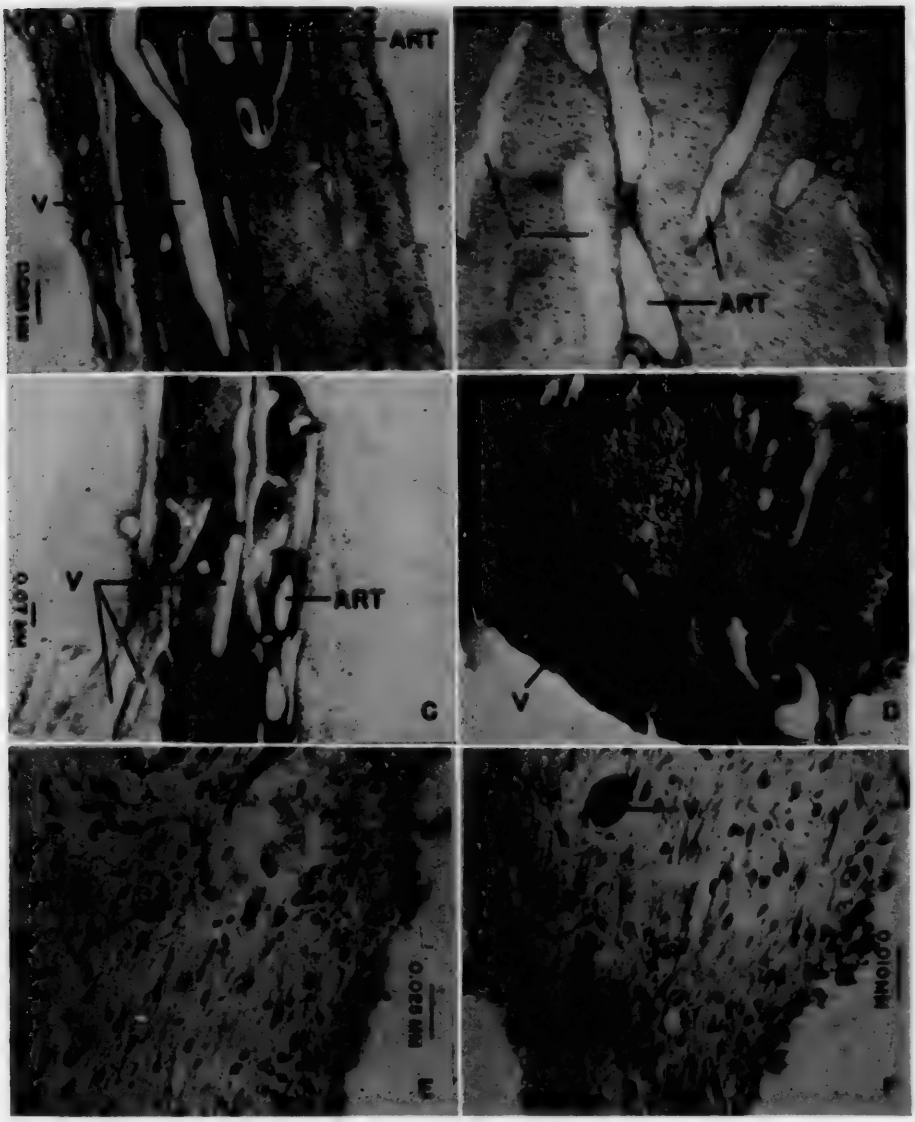

Fig. 2.—*A*, Photomicrograph of the mid-region of a longitudinal section of human tooth pulp (bicuspid), showing large diameters of vein and artery.

B, Photomicrograph of mid-region of a longitudinal section of human tooth pulp (bicuspid), showing large diameters of blood vessels.

C, Photomicrograph of mid-region of longitudinal section of canine tooth pulp (incisor), showing large diameters of blood vessels.

D, Photomicrograph of cross section through mid-region of canine tooth pulp (incisor), showing diameters of blood vessels.

E, Photomicrograph of cross section through coronal end of human tooth pulp, showing diameter of vein.

F, Photomicrograph of cross section through apical end of human tooth pulp, showing diameter of vein.

characteristic is especially borne out by the column giving the average measurements. The two striking exceptions to this rule lie in the case of the arteries in the mid-region of the pulps of deciduous teeth, where the average diameter is 0.0605 mm., while the veins in the same region only average 0.0384 mm. in diameter, and in the case of the arteries in the coronal region of the pulps of adult teeth, which measure 0.0325 mm. in diameter, while the veins in the corresponding region average 0.021 mm. in diameter. Possible explanations for these exceptions will be discussed later.

The diameters of the blood vessels are consistently smaller in the pulps of deciduous teeth than those found in the corresponding areas in the pulps of adult teeth. This present study did not attempt to determine the growth rate of the blood vessels in deciduous and/or adult tooth pulps; however, the authors' observations suggest that the most rapid growth rate of the pulpal blood vessels is concurrent with the growth of the teeth, hence little change in growth size occurs after the teeth have reached their maximum size.

DISCUSSION

Provenza[7] is the only worker to have previously reported this unusual condition of the enlargement of the blood vessels; however, in the earlier paper he did not concentrate on this phase of study. In the same paper, he noted that the histologic composition of the main blood vessels and that of their branches are identical, even in the thickness of the walls; the sole difference rests in the size of the vessels.

This spindle-shaped condition of the veins and arteries may be interpreted as the familiar "conical" geometry of vessels in which the diameters are smaller at the junctions where the branches arise, but become larger shortly after the branching. However, if this interpretation is to be accepted, the conditions as described for the blood vessels of the canine and human tooth pulp represent extreme examples. On the other hand, the artery(ies) entering through the apical foramen are smaller in diameter than those in the mid-region of the pulp, and since several branchings have occurred (as far as the authors can determine, the exact geometry of vessel anastomosis in tooth pulp has never been reported), the increase in size of these arteries cannot be directly attributed to the "conical" appearance of those main arteries entering through the foramen.

In regard to the arteries being larger than the veins in the mid-region of the pulps of deciduous teeth, this observation might be interpreted as suggesting a greater demand for arterial circulation in the mid-region, which may be correlated with a greater degree of metabolic activity. Ensuing histochemical studies conducted in this laboratory may contribute further evidence to support this postulation. The same concept may well hold true in the case of the arteries found in the coronal extremity of pulps in adult teeth, which are greater in diameter than the veins located in the same region.

From our understanding of arteriovenous circulation, as has been shown,[10, 11, 12] the walls of the vessels are permeable and can be actively engaged in the exchange of molecules. Hence, it appears logical to conclude that, in

both dog and human tooth pulp, increased vascularity is accomplished by two mechanisms: (1) by the anastomoses of existing blood vessels to supply the greater tissue areas, and (2) by the enlargement of the diameters of the blood vessels to increase the surface contact areas.

From an anatomic standpoint, it is of some interest to note that the blood vessels of canine and human pulps are not consistently decreasing in diameter, as is the usual case, but possess medial areas which are greater in diameter, hence a spindle-shaped inflorescence.

SUMMARY

The tooth pulps from incisors of dogs and bicuspids of human beings were studied histologically. In all cases, the veins and arteries seen in the mid-region of the pulps were greater in diameter than those observed in the apical and coronal extremities. The dimensions of these blood vessels were given. The diameters of the veins were generally greater than those of the arteries found in a comparable region. It was suggested that there may be a need for greater arterial circulation in regions where the arteries are greater in diameter than the corresponding veins and that a greater metabolic rate may exist. It was proposed that increased vascularity in the pulp is accomplished by the branching of the existing blood vessels and by an increase in vessel diameters.

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ELECTRON MICROSCOPY OF THE AMELODENTINAL JUNCTION DURING EARLY DEVELOPMENT OF THE MOLARS OF HAMSTERS

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THE light microscopy of the amelodentinal junction and early stages of Amelogenesis and dentinogenesis has been fully described.[1, 2, 2a, 3] Previous electron microscopy studies of developing teeth have been confined mainly to the forming enamel and dentin. Sognnaes, Scott, Ussing, and Wycoff[4] and Watson and Avery[5] have reported on developing teeth, but little cellular detail was described. Since then, Nylen and Scott,[6] using mouse incisor, have described some cytologic details. In recently differentiated ameloblasts, almost all of the mitochondria were found basal to the nucleus and were separated from the cell membrane by a band of dense fibrillar material. These authors state that there was little cellular alteration until the beginning of enamel formation, when the endoplasmic reticulum increased markedly and was arranged more or less parallel to the long axis of the cell. In a recent monograph, Scott and Ussing have described in detail the electron microscopy of early dentinogenesis.[6a] Concomitantly with the beginning of differentiation of the odontoblasts, two types of fibrils appear, one type very fine and the other coarser and typical of collagen.

As part of an electron microscopy study of developing teeth of hamsters, the area of the future amelodentinal junction was found to yield considerable information concerning cellular differentiation of the dental formative cells.

Several differences from conventional light microscopy were found and will be described.

MATERIALS AND METHODS

Newborn hamsters were used because of the ease with which the developing molars could be dissected, and the histologic similarity of the fully formed teeth with those of human beings.

Under a dissecting microscope, vertical incisions were made in the molar areas of hamsters 2 to 6 hours old. The tooth germs were identified and 1 per cent buffered osmium tetroxide (pH 7.2) poured on the tissue. After 10 minutes, the tooth germs were carefully dissected, cut into small pieces and placed in the same fixative for a further 2 hours, rinsed in water for half an hour, and dehydrated in increasing strengths of ethyl alcohol. The tissue was then embedded in methacrylate, and thin sections cut on a Porter-Blum microtome at 200 to 300Å.

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Electron micrographs were made with a Philips EMU 100A at 60 kv. (To illustrate certain features completely, it was found advisable to combine electron micrographs of adjacent areas. Such montages are indicated in the legends.)

No decalcification or shadowing technics were used. The blocks were easily sectioned with the exception of a few first molars. Here calcification was so advanced as to cause shattering. Some of the blocks containing calcified tissue, however, could still be cut with ease.

RESULTS

The first area found shows the epithelial cells of the internal enamel epithelium lined up in a uniform row, below which is the primitive pulp. Fine fibrils can be seen originating from the primitive pulp and running toward the interface between epithelium and connective tissue. This is the first indication of the future amelodentinal junction (Fig. 1 and 1*A*).

The next stage is the rapid differentiation of the odontoblasts which causes the disappearance of the cell-free zone[2a] and the formation of a fibrillar layer approximately 1μ thick on the dentinal side of the interface (Figs. 2 and 3). This layer is composed of fine fibrils which arise from the pulp. The primitive ameloblasts are separated from this fibrillar layer by an intact amelodentinal interface. The odontoblasts are also separated from this membrane but the separation is incomplete, being formed by the continuation of the cell membranes of the odontoblasts, giving rise to T-shaped structures (Figs. 2, 3, 4, and 4*A*). Since banding of the fibrils was not observed, and therefore the fibrils could not be considered collagen, it was felt that this area could not be considered predentin but did resemble the amelodentinal membrane seen in light microscopy.

The ameloblasts have large nuclei which almost fill the cytoplasm. Spherical or oval bodies are found in the basal portion of the cell. It is felt that these are mitochondria, perhaps in a very early developmental stage.

Endoplasmic reticulum and mitochondria can be seen in the odontoblasts at this time. Prominent nucleoli are found in the nuclei of both the ameloblasts and the odontoblasts (Fig. 4).

The interface between the basal end of the ameloblasts and the amelodentinal membrane now becomes discontinuous (Figs. 4 and 4*A*) and finally disappears (Fig. 5).

Electron dense material invades the membrane from the pulpal side (Fig. 5). This has been shown to be due to the inclusion of further organic material into the fibrillar material prior to calcification.[6a] The next change noticed is the change in position of the nuclei to the ends of the cells furthest from the membrane while the base of the ameloblasts becomes vacuolated.

The ameloblasts become columnar (Fig. 6). The mitochondria are situated at the distal end of the cell around the nucleus, and nucleoli are very prominent. The structure of the now mature ameloblasts is very complicated. Cell membranes are extremely difficult to discern, except between the distal end of these

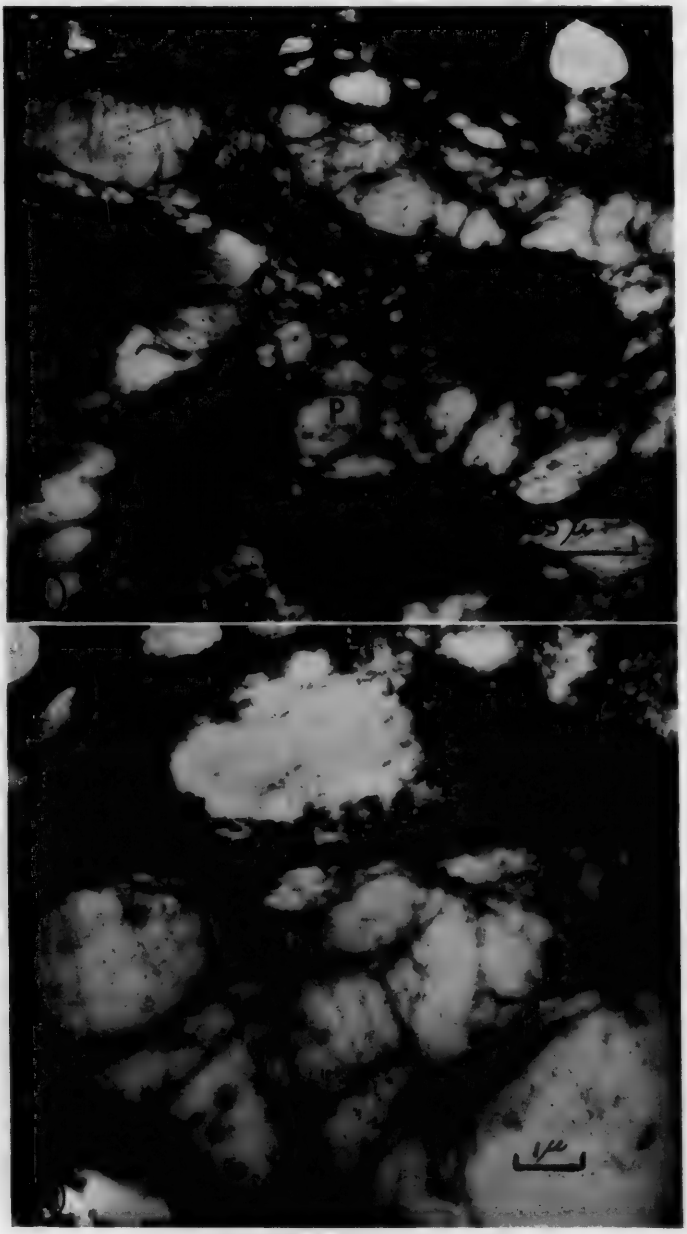


Fig. 1.—Amelodentinal junction in a very early stage. Delicate fibers from the pulp (*P*) running toward the interface between the future ameloblasts and the primitive pulp.

Fig. 1*A*.—Higher magnification of part of Fig. 1, showing the double nature of the interface and the cell-free zone.

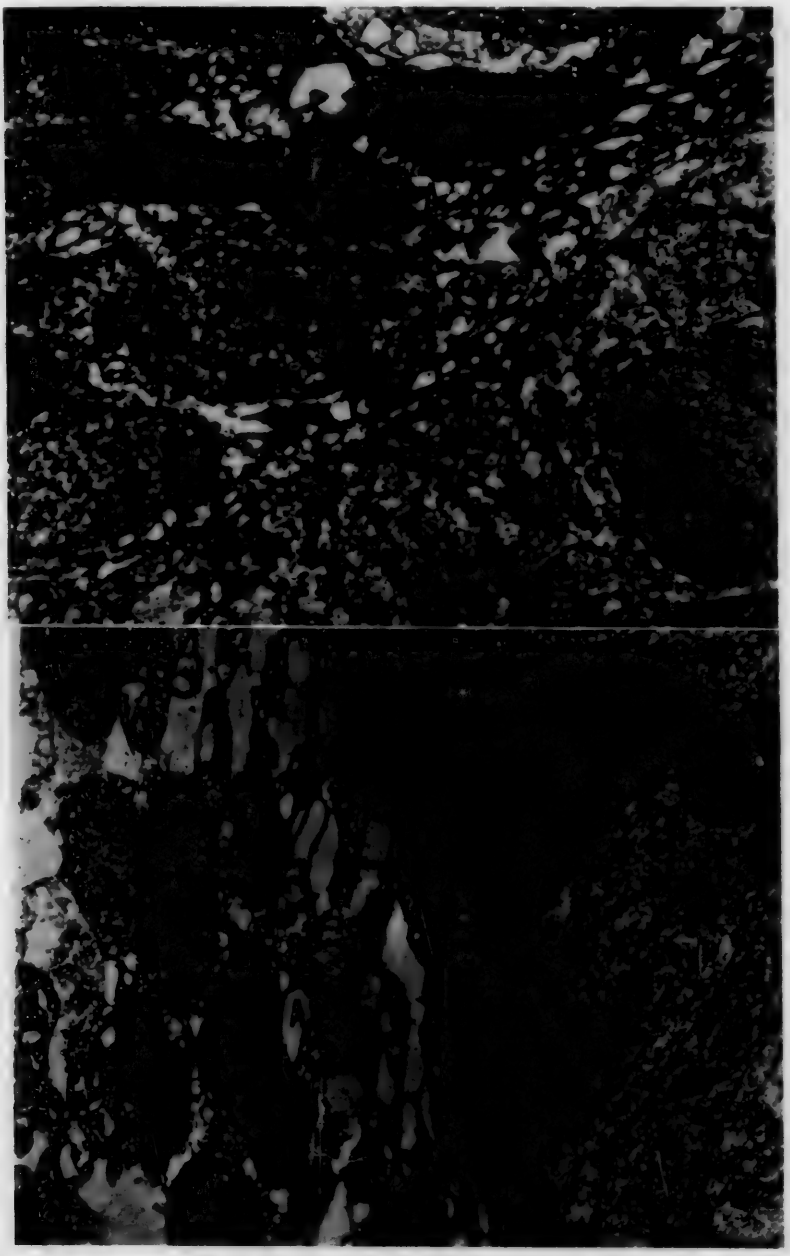


Fig. 2.—Disappearance of the cell-free zone. Differentiation of odontoblasts (*O*), and the presence of endoplasmic reticulum and mitochondria in these cells. The ameloblasts (*A*) are still undifferentiated, but mitochondria are now present.

Fig. 3.—The amelodentinal membrane (*M*) is now much more evident as is the differentiation of the odontoblasts (*O*). The ameloblasts (*A*) are still undifferentiated.

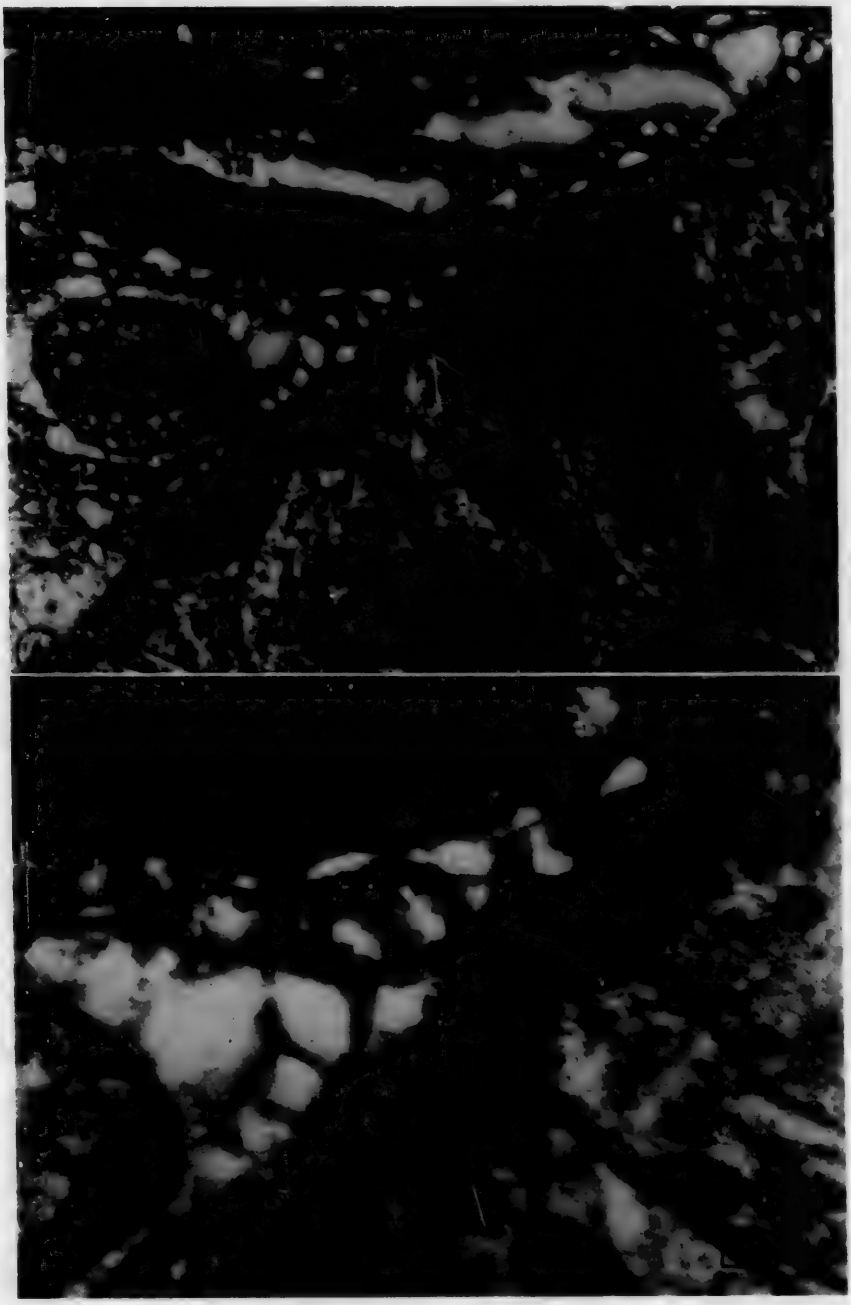


Fig. 4.—The amelodentinal membrane is now well formed. The double membrane separating the ameloblasts from the membrane shows discontinuity at points marked with arrows. The T-shaped so-called terminal bars of the odontoblasts are also shown (*T*).

Fig. 4*A*, Higher magnification of Fig. 4.

cells and stellate reticulum. Fig. 6 also shows endoplasmic reticulum disposed both at right angles and vertical to the forming matrix. A small amount of matrix has been formed. There is no sharp demarcating line between the cytoplasm and the matrix, which seems to develop from granular cytoplasmic extensions. The cytoplasm of the ameloblasts themselves contains fine granules and coarse osmiophilic "strings" stretching from nucleus toward the matrix (Figs. 6 and 7). As the odontoblasts retreat further, the amelodentinal membrane becomes blended with and indistinguishable from predentin (Fig. 7).

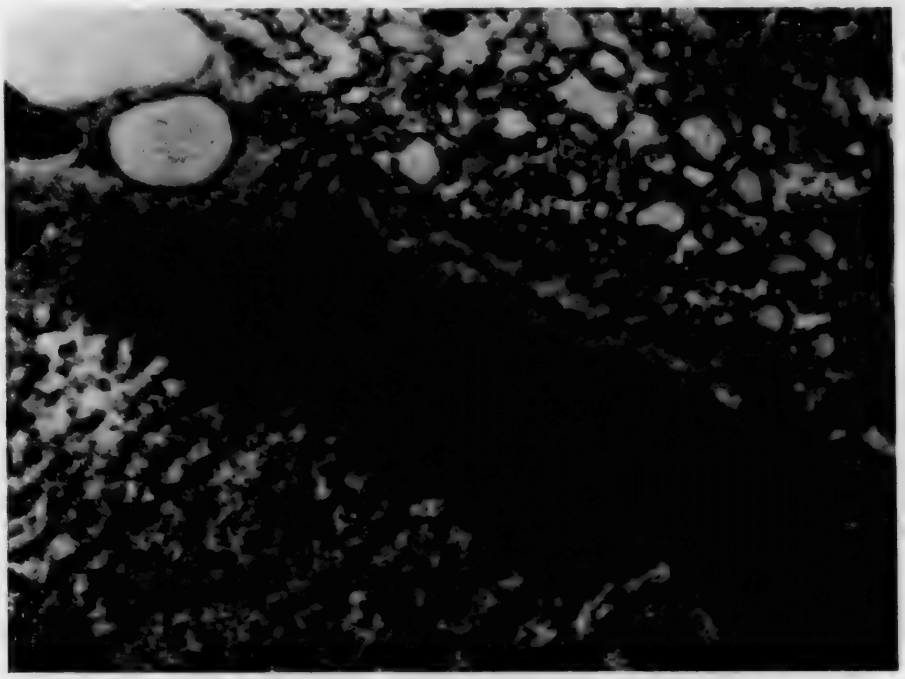


Fig. 5.—Amelodentinal junction at the beginning of calcification. Base of ameloblasts (*A*) and odontoblasts (*O*). Dense osmiophilic material is seen invading the membrane (*M*) from the dentinal side.

Figs. 8 and 9 show the odontoblasts with the endoplasmic reticulum and mitochondria situated between the nucleus and the now electron dense membrane. The endoplasmic reticulum runs parallel to the long axis of the odontoblast.

The cytoplasm of the odontoblasts is clear compared with that of the ameloblasts as seen in Figs. 6 and 7.

The amelodentinal junction is now established. Further description of forming enamel and dentin is considered outside the scope of this paper and will be reported separately.

DISCUSSION

The first indication of the amelodentinal junction appears as a double membrane forming an amelodentinal interface, followed by the formation of a

fine fibrous layer, formed before any differentiation of the epithelium and connective tissue. The loose arrangement of the connective tissue accounts for the so-called cell-free zone seen in light microscopy.[2a] Subsequent thickening of the membrane, and the differentiation of odontoblasts, rapidly fills up this area.

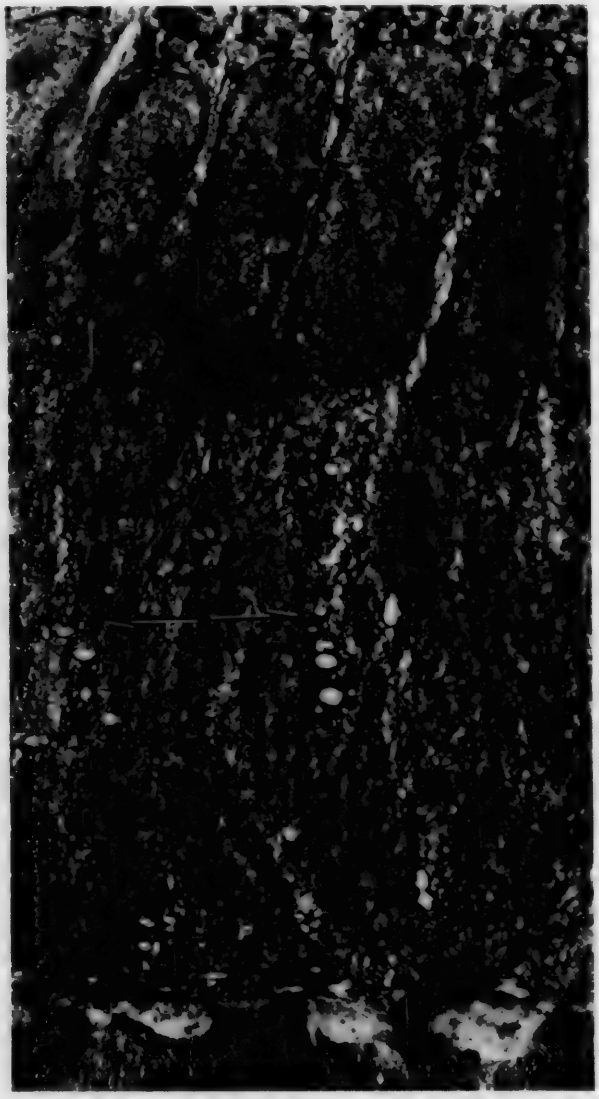
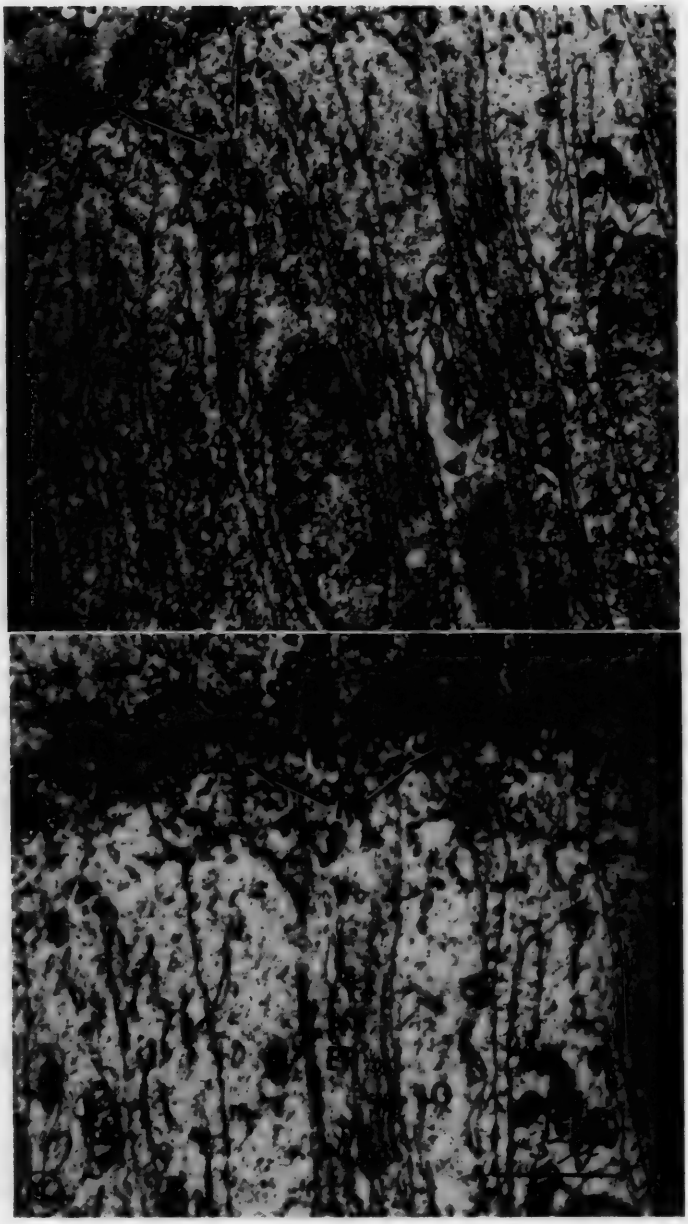


Fig. 6.—(Montage.) Mature ameloblasts. A small amount of enamel matrix (*E*) has been formed and the cytoplasmic extensions, presumably "Tomes's processes," are visible at basal end of the ameloblasts. Osmiophilic "strings" (*S*) are seen stretching from the nucleus toward the forming matrix. Endoplasmic reticulum (*ER*) runs mainly at right angles to the long axis. Mitochondria (*M*).

Fig. 7.—(Montage.) The amelodentinal membrane is now calcified and predentin (*PD*) can be seen between the odontoblasts (*O*) and the membrane. The irregular border between the enamel matrix (*E*) and the membrane (*M*) can be seen.



Figs. 8 and 9.—Odontoblasts (*O*) showing the endoplasmic reticulum (*ER*) mainly parallel to long axis of the cell. Note the relatively clear cytoplasm as compared with the ameloblasts shown in Figs. 6 and 7. Membrane (*M*).

This seems a more logical explanation than the elongation of the ameloblasts toward the dentinal papillae. Odontoblasts begin to develop long before the ameloblasts become columnar. As pointed out by Mummery[7] (referring to the edentata which have no enamel), ectoderm, and not differentiated ameloblasts, is essential for odontoblast formation. This is also true of the epithelial sheath of Hertwig during root formation. The incomplete boundary between the odontoblasts and the basement membrane coincides with the terminal bar apparatus of light microscopy. Watson and Avery found an interrupted line, while these micrographs show T-shaped double structures, the vertical limb being the cell membrane. Perhaps different fixation technics could account for these discrepancies. No intercellular bridges were found between odontoblasts. The breaking up of the double membrane at the basal end of the ameloblasts would allow a limited penetration of the enamel by the dentin,[7] and would account for the formation of the so-called enamel spindles.

The complicated structure of mature ameloblasts requires more study. The consistent failure to demonstrate distinct cell membranes between these cells supports Widdowson,[3] who felt that, although such cell membranes have been described, further proof of their existence was needed. The idea of the inner enamel epithelium being a syncytial-like structure is an intriguing one, and raises the question of whether one ameloblast forms one enamel rod. The granular area at the base of the ameloblasts corresponds to Tomes's process of conventional light microscopy. However, there is supposedly one process per cell, and this can be easily verified by counting processes and nuclei. In the electron micrographs there are more nuclei than processes, but difference in thickness in light and electron microscope sections must be borne in mind.

Further studies are being carried out on the developing matrix of enamel, with special reference to rod formation.

SUMMARY

1. Electron micrographs were prepared of developing molars of newborn hamsters.

2. First indication of amelodentinal junction is a very delicate interface between the enamel epithelium and the odontoblasts.

3. An amelodentinal membrane forms on the dentinal side of the interface and odontoblasts differentiate before the ameloblasts become columnar.

4. Forming ameloblasts are separated from the fibrillar amelodentinal membrane by a complete double cellular membrane which later breaks up and disappears. Between the odontoblasts and the amelodentinal membrane, however, the boundary is incomplete, and this is tentatively identified with the so-called terminal bar apparatus.

5. Preliminary observations on the older ameloblasts suggest a syncytial-like arrangement.

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THE DIFFUSION OF GLUCOSE THROUGH MICROBIAL PLAQUES

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THE characteristic decalcification of dental enamel in the carious process is presumably caused by acids formed in or under the dental plaques.[1] These acids are derived from dietary sugars or other carbohydrates by the normal enzymatic glycolytic reactions caused by microbial enzymes.[2, 3]

It has been quite adequately demonstrated that dental caries is not prevalent unless refined sugars are readily available. Furthermore, experimental animal caries has not been produced in the absence of dietary sugar. When the cariogenic diet is given by stomach tube no caries results.[4] Cariogenic diets fail to cause caries in the absence of microbial enzymes as demonstrated in germ-free animals.[5, 6]

It has been further demonstrated that acids form rapidly when fermentable sugar and cariogenic foods are applied to caries-active dental plaques in situ,[7-10] but acid potentials sufficient to decalcify the teeth do not occur when sugar is applied to the dental plaques of caries-inactive individuals.[11, 12]

Even though it would seem that the relation of sugar to dental caries has been adequately demonstrated, there are a few factors that ostensibly do not conform. Most fruits and berries contain about 10 per cent fermentable sugar. Many primitive people live almost entirely on fruits and berries for considerable periods of time, yet these individuals do not get many carious lesions. When animals are given acidulated 10 per cent sugar solutions instead of drinking water, they ordinarily do not get dental caries, although a generalized acid decalcification does occur.[13, 14]

There is one characteristic that is common to all fruit juices, soft drinks, and the like which is not common to candy and many confections. The latter are made of, or contain, sugar in concentrated form whereas the former contain sugar in relatively dilute concentration.

According to the chemo-parasitic theory, the acids formed by the degradation of the sugars must be under the plaque in contact with the tooth before decalcification can occur. In view of this, in order for sugar or acid to be harmful, either the sugar, the acid, or both must penetrate the plaque to the

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tooth surface. Among the factors that govern the rate of penetration is diffusion pressure. This is a function of the molecular concentration $P = \frac{g}{M} RT$.

There have been no attempts to determine quantitatively the rate of penetration of sugar into microbial plaques, although attempts of a qualitative nature have been made.[15] This paper deals with the quantitative determination of the rate of penetration of sugar into synthetic microbial plaques under various concentrations or diffusion pressures.

EXPERIMENTAL PROCEDURE

Approximately 60 ml. of paraffin stimulated caries-active saliva was collected and glucose added to a concentration of 5 per cent. The saliva-glucose mixture was then incubated at 37.5° C. for 24 hours, after which it was centrifuged and the clear supernatant drained off. The sediment was then washed with 0.9 per cent salt water until the pH was 6.5 to 7.0. It was found by experiment that this method provided a microbial sediment that was relatively uniform and could be duplicated. Unless the saliva was incubated with sugar, the salivary sediment was not uniform and there was considerable variation in lactate production. The thick microbial paste was then placed in a paraffin cell (Fig. 1) and vibrated until a dense mat, 1 cm. in area and 1 mm. thick, was formed.

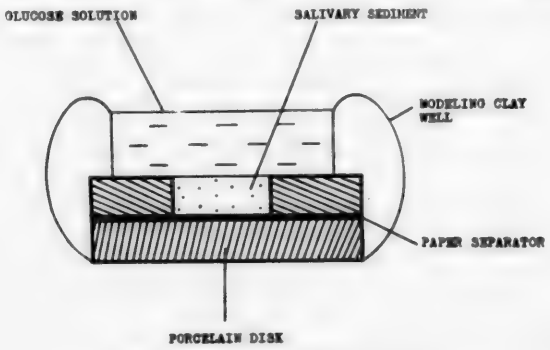

Fig. 1.—The fermentation cell.

Any excess material was removed, the wall of the cell surrounded by a layer of modeling clay, and the well thus produced was filled with the test solution of glucose. Sugar concentrations of 0.1 M, 0.3 M, 0.8 M, and 1.0 M were used and allowed to remain in contact with the plaque for intervals of 5, 15, and 30 minutes. The cell was then incubated at 37.5° C. for the desired length of time, after which the sugar solution was blotted off with filter paper and the cell then plunged in a dry-ice acetone mixture. The modeling clay was stripped from the cell and the paraffin walls were then trimmed, after which it was attached in the frozen condition to the specimen holder of a microtome. This was accomplished by means of a drop of water, which was then frozen by means of a stream of liquid carbon dioxide. The specimen was kept frozen throughout

the procedure by means of a stream of liquid carbon dioxide. A slice of approximately 0.025 mm. was removed and discarded. This provided a smooth surface so that all subsequent slices would be of uniform thickness. The 1 mm. cylinder was then successively sliced into ten samples, each 0.1 mm. thick. All the samples were therefore of equal volume. Each sample was then transferred, still in the frozen state, to a 10 ml. beaker by means of a camel's-hair brush. Each sample was kept in an ice bath to prevent further fermentation. The expansion of the mass on freezing provided ample protection for the blade when 10 slices, 0.1 mm. thick, were removed. Each sample was then analyzed. In one set of duplicate series each slice was analyzed for glucose. In another duplicate series, the first, third, fifth, seventh and ninth slices were analyzed for glucose while the even-numbered slices were analyzed for lactate.

THE DETERMINATION OF GLUCOSE

Glucose was determined by the method of Rupe and Farmer.[16] Two milliliters of distilled water was added to each slice of microbial cylinder. The sample was well mixed after which two 1 ml. aliquots were removed for duplicate analyses. Each sample was then deproteinized by the addition of 0.2 ml. 7 per cent copper sulfate, $CuSO_4 \cdot 5H_2O$, and 0.2 ml. of 10 per cent sodium tungstate, $Na_2WO_4 \cdot 2H_2O$. The samples were then centrifuged and the supernatant of each transferred to a 20 by 150 mm. test tube. Each sample was then made alkaline to phenolphthalein by the addition of dilute NaOH after which one milliliter of copper reducing agent was added. Each tube was then capped with aluminum foil and heated in a steam bath for 10 minutes and then cooled in running water. Two milliliters of phosphotungstate reagent was then added and the color allowed to develop. The colored solution was then quantitatively transferred to a 25 ml. volumetric flask, diluted to the mark, and well mixed. The transmittance of the colored solution was then determined by means of a Coleman Universal spectrophotometer at a wave length of 725 mμ, using a red filter. The blank in each case was a sample of distilled water subjected to the same procedure. The amount of glucose was determined by comparison with a previously prepared standard curve.

Reagent I—Alkaline Copper Tartrate Reagent.—Dissolve 28 grams of anhydrous disodium phosphate and 40 grams Rochelle salts, (sodium-potassium tartrate) in about 700 ml. of H_2O, add 100 ml. of 1N NaOH and mix until dissolved. With stirring add 80 ml. of 10 per cent cupric sulfate solution ($CuSO_4 \cdot 5H_2O$). Mix well. Finally, add 180 grams anhydrous sodium sulfate, dissolve, and dilute the resulting solution to 1 liter. Allow to stand for a day or 2 during which time a slight sediment will settle. Filter the solution through a fine grade filter paper. This reagent keeps indefinitely.

Reagent II—Sodium Tungstate Reagent.—Dissolve 100 grams sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) (molybdate free) in 150 ml. H_2O in a 250 ml. ground glass round bottom flask. Add 33 ml. of 85 per cent orthophosphoric acid. Boil under reflux with a water-cooled condenser for one hour. Cool to room temperature. If the solution is deeply colored, decolorize by adding enough bromine water to produce an orange color. After a few minutes boil off the excess bromine. Cool the solution and dilute to 500 ml. This reagent keeps indefinitely.

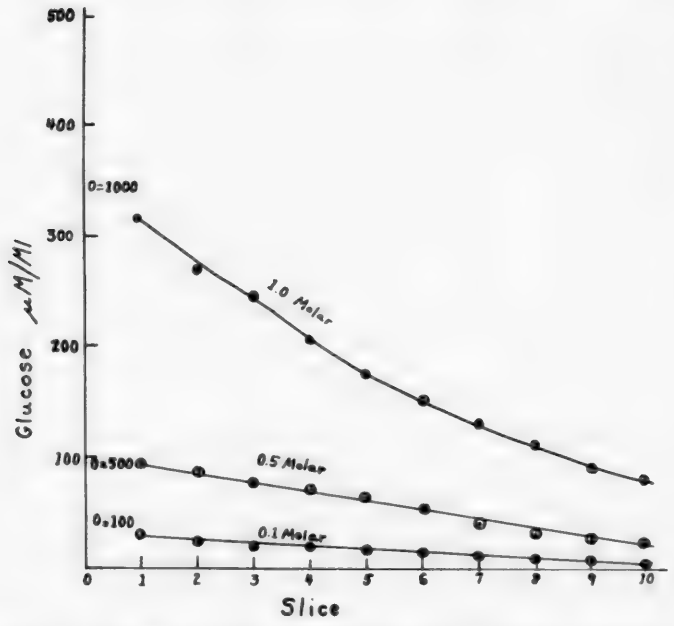

Fig. 2.—The sugar content of each layer after 5-minute incubation.

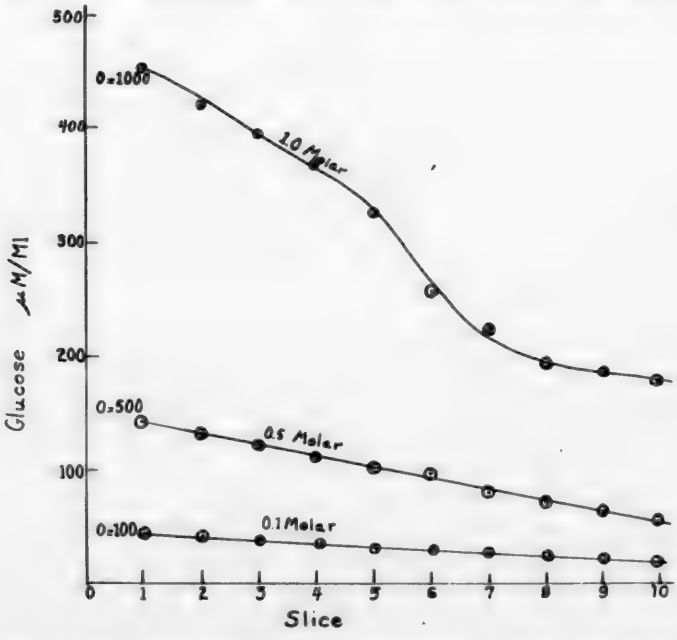

Fig. 3.—The sugar content of each layer after 15-minute incubation.

RESULTS

The results are presented in Table I and in Figs. 2, 3, 4, and 5. The differential between the concentration of the sugar solution and the first layer of the microbial membrane is rather large with all concentrations and at all time intervals up to 30 minutes. Even at the 30-minute interval, the outer

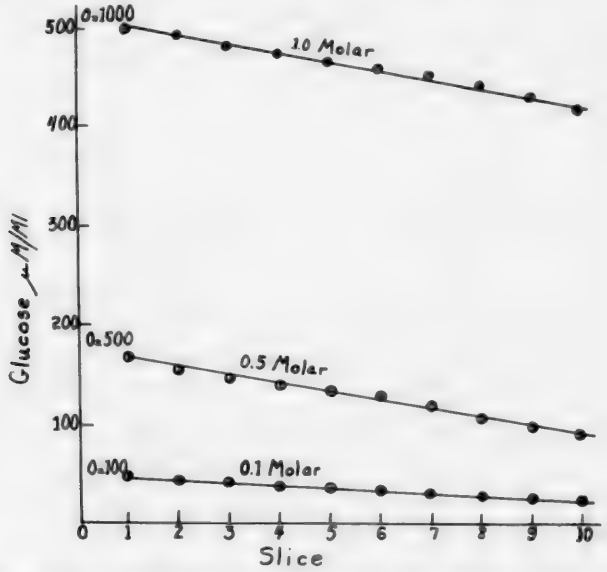

Fig. 4.—The sugar content of each layer after 30-minute incubation.

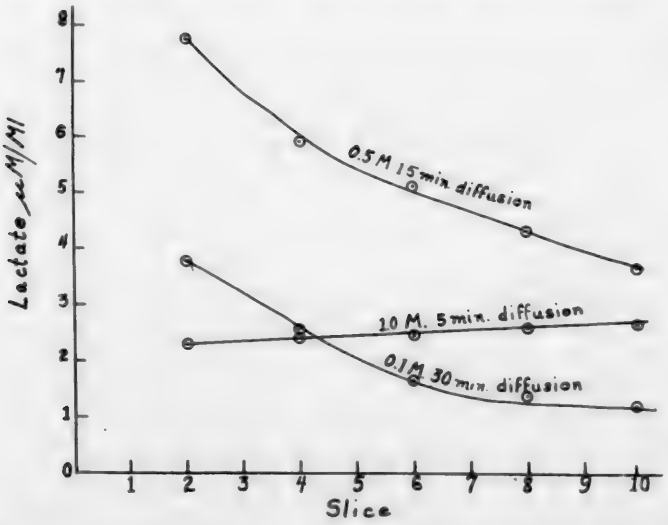

Fig. 5.—The lactate content of each layer after intervals of incubation.

layer of microbial plaque did not approach the concentration of the solution in contact with it. This is true regardless of the volume occupied by solid materials, as these plaques are about 80 per cent water.

TABLE I. CONCENTRATION OF GLUCOSE AT VARIOUS TIME INTERVALS

	TIME (MINUTES)														
	5	15	30	5	15	30	5	15	30	5	15	30	5	15	30
SLICE	MICROMOLS/ML.			MICROMOLS/ML.			MICROMOLS/ML.			MICROMOLS/ML.			MICROMOLS/ML.		
0	100	100	100	300	300	300	500	500	500	800	800	800	1000	1000	1000
1	31	44	49	78	130	142	95	142	167	219	339	424	325	452	515
2	30	42	45	73	110	131	88	132	149	170	292	396	276	416	493
3	30	40	43	67	95	125	85	122	145	161	280	378	246	399	490
4	30	37	40	62	78	123	83	113	143	157	272	339	215	376	478
5	29	31	37	57	69	120	71	101	141	153	265	331	178	323	468
6	25	28	35	49	62	111	52	88	140	148	255	320	152	258	464
7	22	27	33	37	54	95	40	79	130	138	237	314	133	215	456
8	20	26	31	28	49	88	33	72	109	120	218	306	113	195	443
9	18	25	30	25	45	81	29	62	98	95	197	290	92	190	429
10	17	25	28	21	42	78	27	52	89	71	177	273	71	182	410

The 0.1 M solution did not penetrate to the bottom layer in a concentration sufficient to form appreciable amounts of lactic acid. Even though the microbial plaque resisted the entrance of sugar into it, once the sugar had entered, the rate of diffusion was roughly proportioned to the molar concentration. The lower layers of the 1 mm. microbial plaque exposed to 1 M solutions for 5 minutes contained roughly the same amount of sugar as the top layer of the plaque exposed to a 0.5 M solution for an equal period of time.

The fermentation to form lactic acid was not consistent in each concentration. When the plaque was exposed to the more dilute solutions, there was insufficient lactate formed for accurate analysis during the short incubation periods. However, if allowed to incubate a full 30 minutes, appreciable amounts were formed in the outer layers. The 0.5 M solutions formed over 7 μM per milliliter on the outer layers but the inner layers contained considerably less. When the 1.0 M solutions were permitted to ferment only 5 minutes, very little fermentation occurred on the outer layers but the concentration of acid increased slightly with depth. The inner layer, after the 5-minute incubation in contact with the microbial plaque, contained more lactate than the inner layer of the membrane which had incubated for 30 minutes in contact with the 0.1 M solution.

DISCUSSION

If one assumes that a plaque or mat made from caries-active microbial sediment is similar in composition and physical characteristics to natural dental caries-active plaques, then there are several apparent discrepancies in the chemo-parasitic theory of dental caries that may be explained. There are many points of similarity between these microbial mats and the normal dental plaque but they are not natural dental plaques and any similarity to them may be purely coincidental. Whether or not they bear any real relation to normal dental plaques, the permeability is similar to the natural ones, as was qualitatively measured by Dobbs.[15]

Even though it has been established that one of the important variables associated with the penetration of sugars into the plaque material is the concentration of sugar in contact with the plaque, there is at least one factor that appears to require more study. The concentration of sugar per volume of plaque in the layer contiguous to the pure solution is much less than we would expect. These microbial mats, and plaques scraped from teeth, are approximately 20 per cent solids and hence one might expect a 20 per cent differential. However, even when the solution is in contact for 30 minutes, a differential of the order of 50 per cent is observed. This discrepancy is probably associated with the permeability of the cell membrane, as it is quite probable that most of the water of the mat is intracellular water. Regardless of the reasons for the discrepancy, the results obtained indicate that high concentrations of sugar on the surface of the plaque are necessary for the diffusion of sugar through 1 mm. of plaque in sufficient quantities to form detectable amounts of lactate.

The differences between natural and refined sugar are dramatically presented. Natural sugars as they exist in sugar cane juice, citrus fruit juices, or any other natural juice are rarely over 0.3 M in respect to sugar concentration. Thus, on the basis of the rate of diffusion of 0.3 M sugar into the 1 mm. mat, one might expect that fruit juices or other dilute sugar solutions, although they may furnish sufficient nutrient to maintain an acidogenic microbial flora, should not produce sufficient acid under a well-formed dental plaque to be of much consequence in the decalcification of teeth. Soft drinks and the like should fall in this category, providing the carbonation does not alter the penetration of the sugar. The acids formed on the surface of the dental plaques should be rapidly neutralized by the saliva if a sufficient amount is present to do so.

The permeability of the dental plaque could well be a factor in regard to caries control. It is quite possible that enzyme inhibitors may be adsorbed on the surface of plaques and prevent all acid formation on the surface while, at the same time, fermentation can proceed as usual in the inner portions contiguous to the tooth. Even in cases where the enzyme inhibitor or the antibiotic is mixed with the cariogenic diet, the sugar may diffuse to the tooth surface and ferment, while the inhibitor or antibiotic inhibits or destroys microorganisms or their enzymes on the surface. This may also help explain the difficulty encountered in sterilizing the mouth.

In view of the resistance to penetration, the dental plaque could well be a natural protection against acid foods and at the same time be a primary link in the sequence of events for the development of a lesion, if it were to become infected with acidogenic organisms.

Whether or not the microbial mat, as described, is the same as the natural plaques, the method is applicable to a wide variety of experiments. The cell may be prepared from natural plaque scraped from teeth and compared with the synthetic variety in respect to the permeability of sugars, fruit juices, and other foods, either free or with enzyme inhibitors added. Furthermore, it may be possible to measure the pH of each layer directly, in addition to the analysis for sugars and lactate. Experiments of this type are now under way.

SUMMARY

The rate of the penetration of glucose solutions of various concentrations through a microbial mat, 1 mm. in thickness, was measured. It was found that, although the mat resisted the rapid penetration of sugar, the rate of penetration was roughly proportional to the concentration. Theoretical aspects of the phenomenon were discussed.

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THE BW1081 MOUSE SALIVARY GLAND TUMOR

A Preliminary Study of Its Growth Characteristics and Host Responses

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THE BW1081 neoplasm is described by the Roscoe B. Jackson Memorial Laboratory of Bar Harbor, Maine,[1] as a myoepithelial cell carcinoma of salivary gland origin which arose spontaneously in one of their laboratory stock of the BALB/c mouse in 1949. Since that time, the Jackson Laboratory has successfully transplanted and carried the neoplasm in this strain. They have reported that the transplanted neoplasm is palpable 7 days following transplantation, killing the host within 35 to 65 days.

Myoepitheliomas of mouse salivary gland origin have been reported by several investigators. Lippincott, Edwards, Grady, and Stewart[2] briefly described transplantable, spontaneous tumors involving the submaxillary salivary glands in mice of strains C and A and suggested that the tumors were of myoepithelial origin. Maver and Dunn[3] reported a transplantable myoepithelioma of mouse salivary gland origin and observed an associated splenomegaly and extensive extramedullary myelopoiesis in the spleen. Andervont and Dunn[4] reported seven spontaneous myoepitheliomas in 212 strain C mice which were under observation for a mammary tumor-agent. Law, Dunn, and Boyle[5] gave a morphologic and histologic description of myoepitheliomas which developed in the parotid gland region of their laboratory stocks of the C and A strain mice and reported an associated granulocytic leukemoid reaction to the transplanted tumors.

Although Bateman[6] has reported a study of certain growth characteristics of and host responses to three myoepitheliomas of mouse salivary gland origin, and Dunham and Stewart[7] have listed the BW1081 neoplasm in their survey of transplantable animal tumors, no study of the BW1081 neoplasm has been reported in the literature. The purpose of this report is to summarize our observations of the growth characteristics of the BW1081 mouse salivary gland tumor and of the host responses to the transplanted tumor.

EXPERIMENTAL

Our line of the BW1081 tumor was started from three transplanted tumors which were received from the Roscoe B. Jackson Memorial Laboratory in

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September, 1956. Observations reported here were made on tumor-bearing animals from the eighth to the twentieth transplant generations. Throughout these studies, the male BALB/c mouse served as the tumor host. All transplantations were made by one of three methods: (1) small bits of tumor minced in approximately 0.2 ml. physiologic saline, drawn into a 1 ml. tuberculin syringe, and injected subcutaneously through a 20 gauge needle, (2) small pieces of tumor carried subcutaneously by a pair of iris forceps through a short cutaneous incision subsequently closed by one or two sutures, and (3) small pieces of tumor carried subcutaneously by an 18 gauge trochar. The point of penetration or incision for tumor transplantation was made routinely over the left flank, and the tumor tissue was deposited laterally in the subcutaneous loose connective tissue, approximately midway between the point of entry and the left scapula. Ether was the anesthetic of choice when anesthesia was needed or desired.

After transplantation, periodic measurements of the tumor length, width, and thickness were made by a Boley gauge. A tumor size index was then calculated as the cube root of the representative tumor volume which was determined by the product of the three tumor measurements.

The animals were housed in solid metal cages with wire mesh covers. No more than 5 animals were kept in one cage. The cages contained a bedding of wood shavings; stock laboratory pellets and tap water were supplied ad libitum.

Blood samples for white blood cell counts and blood smears were drawn from the tip of the tail which was clipped by a pair of small, sharp scissors. The tail was gently manipulated, but not squeezed, in order to encourage a sufficient flow of blood. However, only a very small amount of blood was required since routine white blood cell counts were made by using special blood diluting pipettes* for small laboratory animals. All blood dilutions were 1-200. The usual hematologic counting chamber was used for the white blood cell counts. Blood smears for differential counts were stained by Wright's stain.

Tissues taken for histologic examination were fixed in Bouin's solution and stained routinely with hematoxylin and eosin. Some special stains were also applied when indicated.

RESULTS

The transplanted tumor first appeared as a small, firm mass within 7 to 10 days after subcutaneous transplantation. Further tumor growth was accompanied by a rapidly-developing central necrosis which, in an intact tumor, was characterized by a clear, straw-colored, mucoid material surrounded by a caseous-like necrosis subjacent to the viable tumor cells. An intact tumor generally became extremely fluctuant as a result of the accumulation of the central, liquid necrotic material. Occasionally, the tumor surface ulcerated with subsequent drainage of the central liquid necrosis and eschar formation. In older tumors, continued ulceration and eschar formation eventually involved the entire lateral surface of the tumor.

*Arthur H Thomas Co., Philadelphia, Pa.

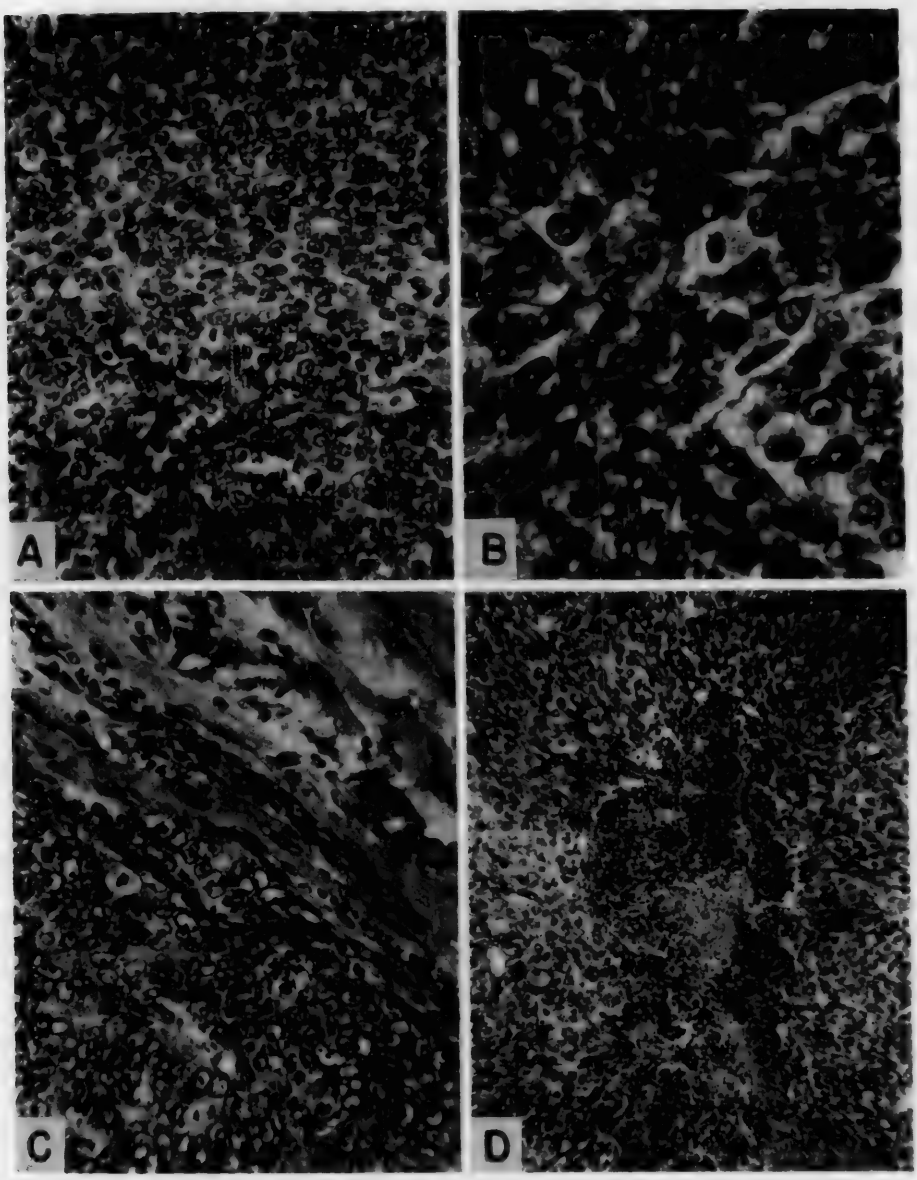

Fig. 1.—BW1081 tumor (hematoxylin and eosin).

A, Section through solid tumor shows typical cell arrangements. Notice the stratification of cells in the center. (Orig. mag. ×250.)

B, Higher magnification shows greater detail of tumor cells. (Orig. mag. ×790.)

C, Periphery of tumor adjacent to muscle mass. Notice the invasion of tumor cells between the adjacent degenerating muscle fibers. (Orig. mag. ×250.)

D, Small area of central necrosis is surrounded by viable tumor cells. (Orig. mag. ×100.)

Histologically, the BW1081 tumor was similar to the myoepitheliomas described by Bateman.[6] Fig. 1 presents four microscopic views of the tumor. The tumor cells were pleomorphic and varied in morphology from elongated, spindle-shaped cells with fusiform nuclei to ovoid cells with vesicular nuclei. The cells were densely packed and often they were arranged in various vague patterns suggesting cord, whorl, and palisade arrangements. The tumor appeared to be divided by thin strands of connective tissue into compartments of irregular size and shape. Frequently, in the outer portion of the central

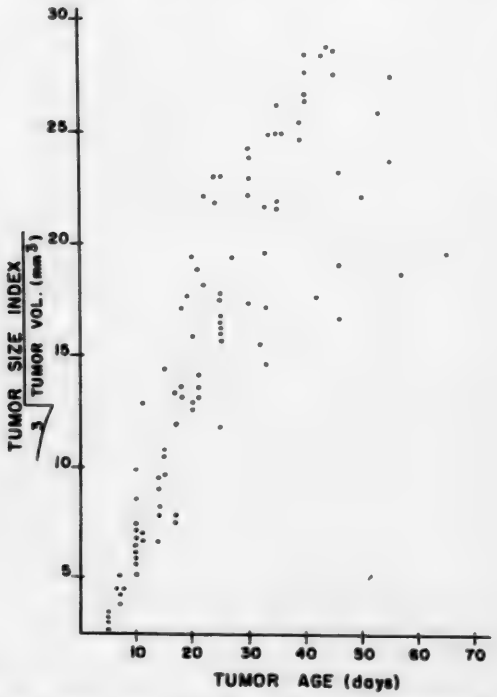

Fig. 2.—Tumor size and tumor age. One hundred and one growth indices for 29 different tumor-bearing mice are plotted against the tumor age in days.

necrotic material, islands of viable cells appeared isolated from the main, peripheral tumor mass. These islands of tumor cells appeared to be aggregates of cells around blood vessels and apparently were attached to the main viable tumor mass.

Fig. 2 illustrates the normal growth-rate pattern of the BW1081 tumor. The growth rate, as expressed by the tumor size index and tumor age, is practically a linear expression up to the maximum tumor size. Maximum tumor size seemed to occur between 35 and 45 days after which the tumor size remained more or less constant until the animal died. However, if ulceration and eschar formation were severe, the tumor sometimes actually diminished in size.

Cachexia and weight loss were experienced by tumor-bearing mice. In comparing the weight of the animal, prior to its receiving the tumor inoculum, with the difference between the weight of the tumor-bearing animal at the time of sacrifice and the weight of the excised tumor, a relative weight loss of between 1 and 4 grams was generally observed. However, one animal, originally weighing 26 grams, weighed 22 grams before the 8 gram tumor which it supported was excised. This animal, therefore, had experienced a relative weight loss of 12 grams.

Deaths of tumor-bearing animals have occurred with 22 to 105 day tumors. However, animals bearing intact or slightly to moderately escharotic tumors generally encountered death between 40 and 50 days following tumor transplantation. The tumors of animals surviving for longer periods of time have not been intact. The entire lateral surface of these older tumors had

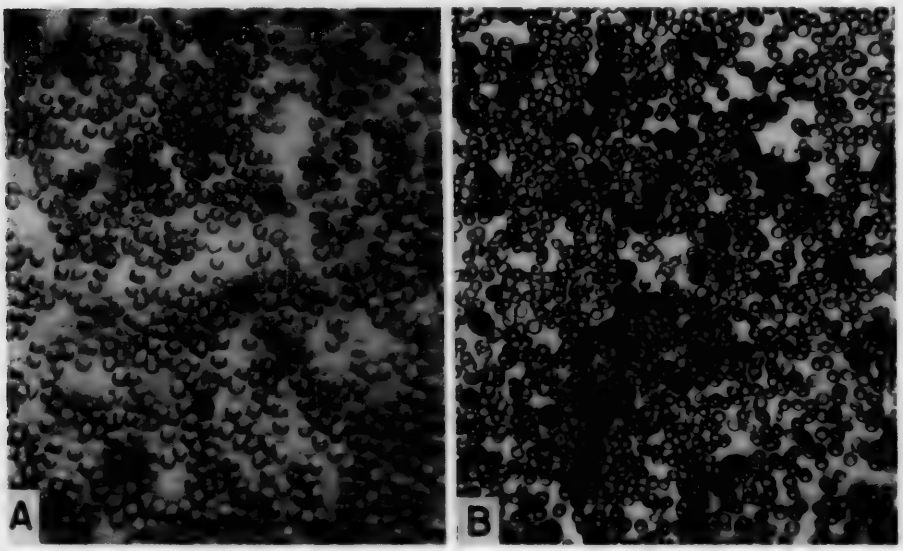

Fig. 3.—Blood smears (Giemsa's stain).

A, Blood smear from control, nontumor-bearing mouse which had a white blood cell count of 8,000 white cells per cubic millimeter. (Orig. mag. ×350.)

B, Blood smear from a tumor-bearing mouse which had a white blood cell count of 250,000 white cells per cubic millimeter. (Orig. mag. ×350.)

become ulcerated with subsequent extensive eschar formation. The viable portion of the tumor existed then as an irregular band of tumor cells lying adjacent to the deeper tissues of the host.

A very definite neutrophilic leukemoid response was a characteristic reaction of the host to the transplanted tumor (Fig. 3). While the normal white blood cell count in mice is quite variable,[8] ranging between 2,000 and 32,000 cells per cubic millimeter, white blood cell counts as high as 740,000 cells per cubic millimeter have been observed in tumor-bearing mice.

In typical differential white blood cell counts, over 80 per cent of the cells have been neutrophilic elements which were predominantly large cells with mature, unbroken ring-form nuclei.[9] Neutrophilic elements normally accounted for only 8 to 25 per cent of the total white cells. In the leukemoid animals, less than 15 per cent of the white cells have been lymphocytes which normally account for 63 to 80 per cent of the total white cells. Monocytes and eosinophils averaged 5 per cent and 2 per cent, respectively, of the total cells. These two values lie within or very near the normal range for monocytes and eosinophils.

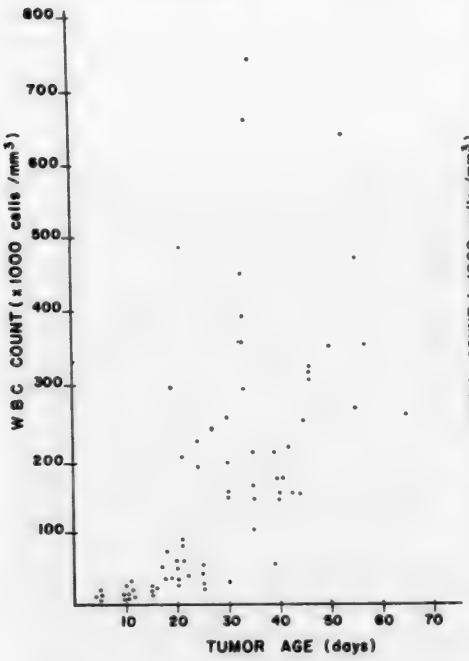

Fig. 4.

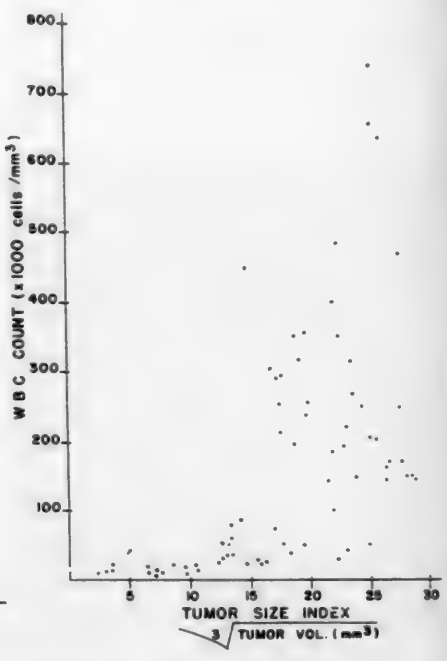

Fig. 5.

Fig. 4.—Tumor age and leukemoid response. Seventy-two different white blood cell counts from 30 different tumor-bearing mice are plotted against the tumor age in days.

Fig. 5.—Tumor size and leukemoid response. Seventy-two different white blood cell counts from 30 different tumor-bearing mice are plotted against the tumor size index.

Correlating 72 different white blood cell counts made from 30 different tumor-bearing mice with the age of the tumor (Fig. 4) suggests only a vague relationship between the severity of the leukemoid response and the age of the tumor. However, in correlating the size of the tumor with the severity of the leukemoid response (Fig. 5), a more direct relationship is observed. The peripheral white blood cell count increases gradually until the tumor approaches a size index of twenty. As the tumor approaches this size, the peripheral white blood cell count usually rises sharply and rapidly to a maximum count which may or may not immediately precede the death of the animal.

Extramedullary myelopoiesis and myeloid infiltration in various organs were constant findings associated with the growth of the transplanted tumor. Varying degrees of myeloid infiltration were seen in the kidneys, adrenals, lungs, and salivary glands. The liver and spleen appeared to be the chief sites of extramedullary myelopoiesis.

In the liver, varying degrees of extramedullary myelopoiesis have been seen primarily around the portal and central vein areas (Fig. 6). However, islands of myelopoietic activity, apart from the major centers of myelopoietic activity around central and portal areas, have been a frequent observation in the liver parenchyma. In severe liver involvement, fibrosis has occurred in some of the more extensive myelopoietic areas. Areas of hyaline-like degeneration have been seen in areas of parenchymal cells which have not been directly involved with the myelopoietic activity.

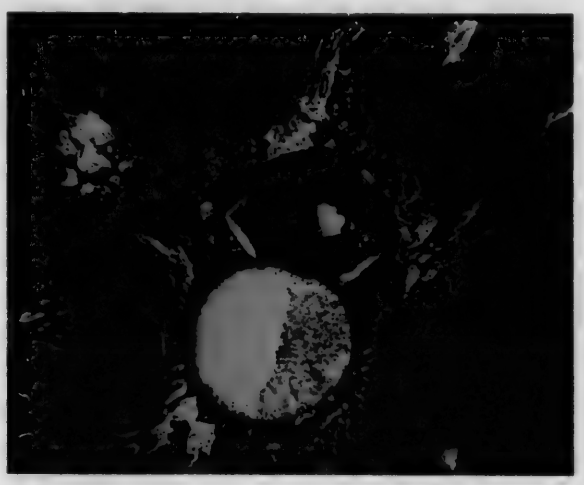

Fig. 6.—Liver from a tumor-bearing mouse. Portal vein area demonstrates extramedullary myelopoiesis. (Hematoxylin and eosin; orig. mag. ×100.)

Splenomegaly was a common but an inconsistent observation. The weights of spleens from tumor-bearing mice varied considerably between a near normal weight of 0.187 gram and 1.105 grams. Little correlation appeared to exist between the size of the spleen and the peripheral white blood cell count. Microscopic alterations which were observed in the spleen involved a modification of the normal architecture of this organ. Although the splenic nodules apparently did not participate in the leukemoid reaction, they became increasingly less distinguishable as the sinus areas became engorged with a predominance of granulocytic forms. Mitotic activity and the number of megakaryocytes were increased considerably (Fig. 7).

A most unexpected alteration was observed in the submaxillary salivary glands of adult, male, tumor-bearing mice. The granular tubules of the submaxillary glands of normal adult male mice are prominent structures composed of large, cuboidal cells filled with a copious supply of a granular-like

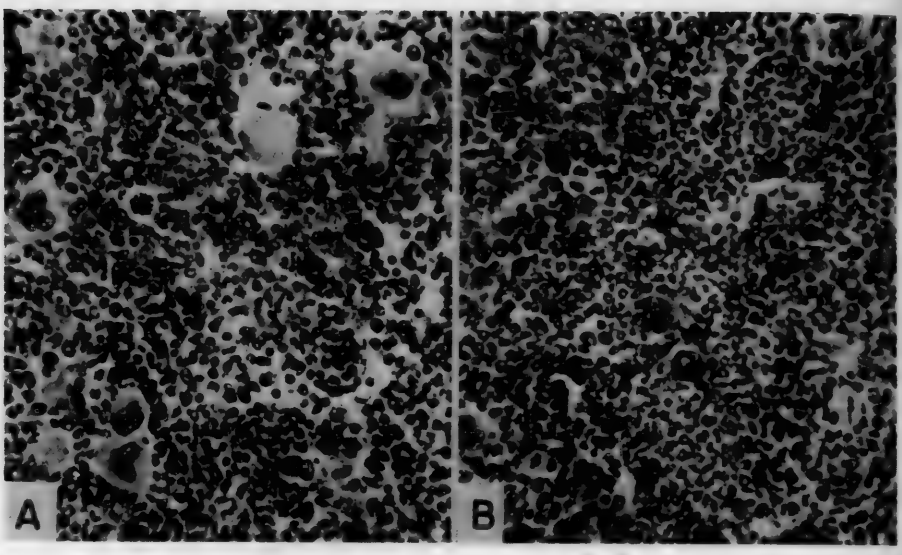

Fig. 7.—Spleen from a tumor-bearing mouse (hematoxylin and eosin).

A, There is extramedullary myelopoiesis with an increase in the number of megakaryocytes and mitotic activity. (Orig. mag. ×350.)

B, Sinus area is engorged with a predominance of granulocytic forms. (Orig. mag. ×350.)

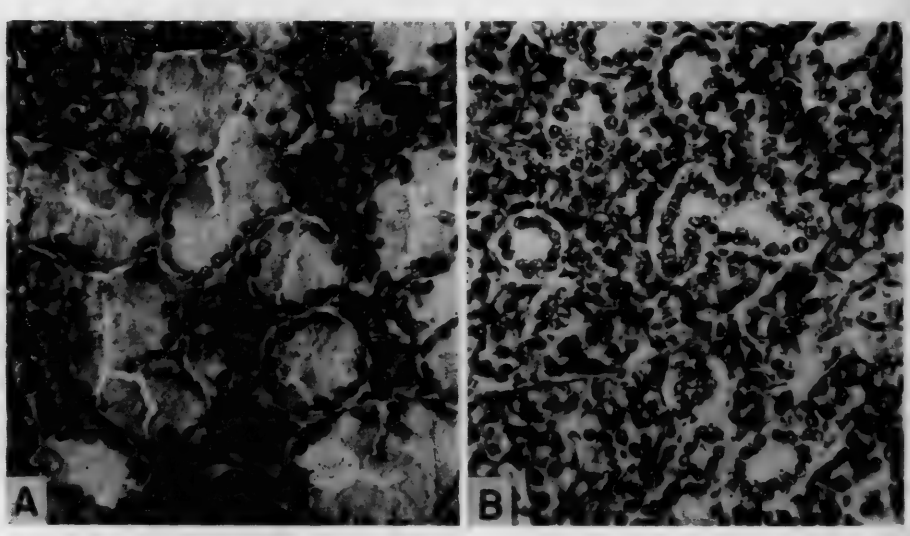

Fig. 8.—Salivary gland (hematoxylin and eosin).

A, Submaxillary salivary gland from an adult male, nontumor-bearing mouse. Note the prominent, granular tubules. (Orig. mag. ×350.)

B, Submaxillary salivary gland from an adult, male, tumor-bearing mouse. Note the absence of granules and the diminution in the size of the tubules. (Orig. mag. ×350.)

substance which stains brilliant pink with hematoxylin and eosin (Fig. 8, *A*). In the submaxillary glands of certain tumor-bearing mice, the granular tubules were remarkably inconspicuous. The cells of these tubules were of a small cuboidal type and appeared to contain very little, if any, of the granular substance. The nuclei almost filled the depleted cells and the scant cytoplasm was only faintly stained (Fig. 8, *B*).

Normal appearing, conspicuous, granular tubules were seen in the submaxillary salivary glands which were removed from one male mouse bearing a 33 day tumor and 8 mice bearing tumors less than 33 days (the white blood cell counts of these 9 mice were between 100,000 and 330,000 white cells per cubic millimeter), as well as in the submaxillary salivary glands which were removed from 3 mice bearing 64 day tumors which had ulcerated severely with subsequent heavy eschar formation (these three mice had white blood cell counts of less than 100,000 cells per cubic millimeter). However, the submaxillary salivary glands which were removed from 24 male mice bearing tumors which were 33 days or older, but less than 60 days old, and having white blood cell counts between 100,000 and 700,000 cells per cubic millimeter, demonstrated inconspicuous granular tubules which were apparently devoid of the normally appearing granular substance.

DISCUSSION

The results of this study indicate that the BW1081 neoplasm is similar in some respects to myoepitheliomas which have been observed and studied by other investigators. The response of the host to the transplanted tumor is probably a true systemic reaction to the tumor, although the mediator, the mechanism, or the significance of this response is unknown. However, the leukemoid response is not specific to the myoepitheliomas since reports of similar responses in mice to mammary tumors and fibrosarcomas are in the literature. Hematopoiesis and splenomegaly have also been reported as responses to a mixed cell sarcoma in the hamster.[10] Whether the same mechanism that is responsible for the leukemoid response is also responsible for the altered granular tubules in the submaxillary salivary glands is a matter of speculation. It is interesting to note that certain experimental endocrine imbalances also produce similar changes in the granular tubules of the submaxillary salivary gland of mice and rats.[11]

SUMMARY AND CONCLUSIONS

1. The BW1081 neoplasm which arose spontaneously in the salivary glands of the BALB/c mouse at the Roscoe B. Jackson Laboratory has been studied.

2. This neoplasm appears to be similar to other myoepitheliomas of mouse salivary gland origin which have been reported.

3. The systemic response to the transplanted tumor includes a leukemoid reaction with a reversal in the normal differential neutrophile and lymphocyte count.

4. Leukemoid infiltration has been observed in the kidneys, adrenals, lungs, and salivary glands.

5. Extramedullary myelopoiesis occurs primarily in the liver and spleen.

6. The severity of the leukemoid response is more directly related to the size and condition of the tumor than to the age of the tumor.

7. A loss of granular substance in the granular tubules of the submaxillary salivary gland of certain male, tumor-bearing mice was found.

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STUDIES ON SALIVARIADENECTOMIZED HUNT-HOPPERT CARIES-RESISTANT RATS

III. Effect of Salivariadenectomy on Dental Caries and Several Groups of the Oral Microflora Under Controlled Feeding Conditions

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A previous study on the Hunt-Hoppert rats has shown that salivariadenectomy increases susceptibility to dental caries.[1] One may assume that interruption of the salivary flow into the oral cavity is the direct cause of this increase in susceptibility. However, it is also logical to assume that several changes in the physiologic or physical behavior of the animal would result following extirpation of the salivary glands. Various changes have been reported in the adrenals and in male and female reproductive organs following salivariadenectomy.[2-5] These changes might influence the development of caries. Rosen, Sreebny, Hoppert, Hunt, and Bachem[6] have shown that a salivariadenectomized rat eats more slowly than a normal rat. Usually, investigators using salivariadenectomized rats have controlled the amount of food consumed by using pair-fed controls. However, since a salivariadenectomized rat spent more time in feeding than unoperated controls, an experiment was designed so that the feeding time, as well as the amount of food consumed, was approximately the same for the operated and normal rats.

EXPERIMENTAL METHODS

Only caries-resistant rats were used in this experiment. The rats were kept on a noncariogenic diet[1] until about 60 days of age. The submaxillary and major sublingual glands were extirpated and a portion of the parotid ducts removed and the severed ends ligated. Following the operation, individual rats were put into wire-bottom cages. They were not able to survive if placed immediately on a restricted feeding regimen. Therefore, the following procedure was instituted. The salivariadenectomized rats were given the noncariogenic diet ad libitum for the first 13 days and then were restricted to 6 continuous hours of daily feeding for 6 days. The latter procedure was continued for 2 days using the HWC† cariogenic ration.[1] Unoperated control rats were pair-fed

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†HWC = Hoppert-Webber-Canniff.

to salivariadenectomized rats during these feeding procedures. On the twenty-second day following the operation, the rats were divided into 3 groups. One group of salivariadenectomized rats was allowed to consume food for 30 minutes three times a day. Unoperated littermates were pair-fed in the same manner. That is, if a salivariadenectomized rat consumed 2 grams of food in the morning, the unoperated control received 2 grams at noon. Another group of salivariadenectomized littermates was pair-fed once a day. That is, the amount of food consumed in three consecutive feeding periods by the first group of salivariadenectomized rats was given all at once to the second group of salivariadenectomized rats on the next day.

The animals were observed for caries in the mandibular molars every 2 weeks. The "caries time" was the difference in days from the date the rat was first given the cariogenic diet to the day dental caries first appeared. Further details of this procedure have been reported previously.[1, 7] The maxillary molars were observed for caries every 4 weeks. Caries time was not determined for these teeth.

Microbiologic studies of several groups of the oral flora were made. The technic of sampling and plating has been described.[1] Examinations for the various groups of oral bacteria were made at 7, 10, 12, 20, and 21 weeks after salivariadenectomy. Samples at 7, 12, and 21 weeks were taken in the morning. Samples at 10 and 20 weeks were taken in the afternoon, because the salivariadenectomized rats that were pair-fed once a day were given their ration at noon. Lactobacilli were determined on two media: (1) Rogosa SL agar, modified slightly by substituting half of the glucose with equal amounts of sucrose and arabinose, and (2) tomato juice agar special (TJA-Difco), containing 0.01 per cent sodium azide. A streptococcal count was made on crystal violet azide blood agar.[1] The numbers of coliforms were determined by the most probable number technic, using lauryl tryptose broth (LTB-Difco). For miscellaneous bacteria, tryptone glucose extract agar (TGE-Difco), containing 4 per cent skim milk, was used.

The experiment was terminated when the animals were about one year old.

RESULTS AND DISCUSSION

The results in Table I show that salivariadenectomy increased the caries susceptibility of resistant rats. Both groups of salivariadenectomized rats developed caries more quickly than unoperated controls. The caries time for salivariadenectomized rats fed 3 times a day was 138.9 ± 11.37 days and for salivariadenectomized rats pair-fed once a day, 140.6 ± 9.12 days, whereas the caries time for unoperated rats was 252.4 ± 13.85 days. The incidence of caries in the maxillary molars was similar to that found in the mandibular molars. The results in Table II show that 71 per cent of the salivariadenectomized rats fed 3 times a day and 50 per cent of the operated rats pair-fed once a day developed caries, whereas none of the unoperated rats developed caries.

TABLE I

EFFECT OF SALIVARIADENECTOMY ON CARIES TIME IN MANDIBULAR MOLARS

GROUP	TREATMENT	NUMBER OF RATS	MEAN CARIES TIME IN DAYS AND STANDARD ERROR	DIFFERENCE IN CARIES TIME	P
A	Salivariadenectomized (fed 3 times per day)	14	138.9 ± 11.37 ←	1.7 ± 14.6	> 0.5
B	Salivariadenectomized (pair-fed to A once per day)	12	140.6 ± 9.12 ←	113.5 ± 17.9	< 0.01
C	Unoperated Control (pair-fed to A 3 times per day)	23*	252.4 ± 13.85 ←	111.8 ± 16.6	< 0.01

*Nine rats had not developed caries at termination of experiment.

These results were similar to those obtained previously when the feeding time was not controlled.[1] Therefore, controlled feeding conditions neither affected the caries time in the mandibular molars nor the incidence of caries in the maxillary molars.

The results of Brewer, Muhler, and Fischer[8] indicated that salivariadenectomy did not increase the caries incidence in maxillary molars. We found that caries developed later in the maxillary molars than in the mandibular molars. The rats used by Brewer and associates were sacrificed at a relatively early age which did not allow this difference to develop.

TABLE II

EFFECT OF SALIVARIADENECTOMY ON CARIES IN MAXILLARY MOLARS

GROUP	TREATMENT	NUMBER OF RATS	NUMBER WITH CARIES	PER CENT WITH CARIES
A	Salivariadenectomized (fed 3 times per day)	14	10*	71
B	Salivariadenectomized (pair-fed to A once per day)	12	6	50
C	Unoperated Control (pair-fed to A 3 times per day)	23	0	0

*Four rats died before termination of experiment without developing caries.

The caries incidence in the maxillary molars of rats following salivariadenectomy has been discussed by Schwartz, Resnick, and Shaw.[9] They pointed out that their experiments with a high sucrose diet produced a greater incidence of caries in the maxillary molars of salivariadenectomized rats than in controls. They stated that Brewer and associates were not able to demonstrate a greater incidence of caries in maxillary molars of salivariadenectomized rats because these investigators used a coarse particle diet. In view of our results, this explanation is incomplete. Actually, the caries incidence in maxillary molars should increase using a coarse particle diet, if the rats are kept on experiment long enough. Therefore, our technic of scoring caries, which is relatively crude, but which necessitates experiments to run longer, helps to explain a difference in results between two groups of investigators.

Restricting the feeding time of rats affected food consumption and weight gain. These data are given in Table III. The gain in weight and amount of

food consumed by salivariadenectomized rats under the controlled feeding conditions of this experiment were considerably less than that found in salivariadenectomized rats fed ad libitum in a previous experiment.[6] The amount of water consumed was about the same.

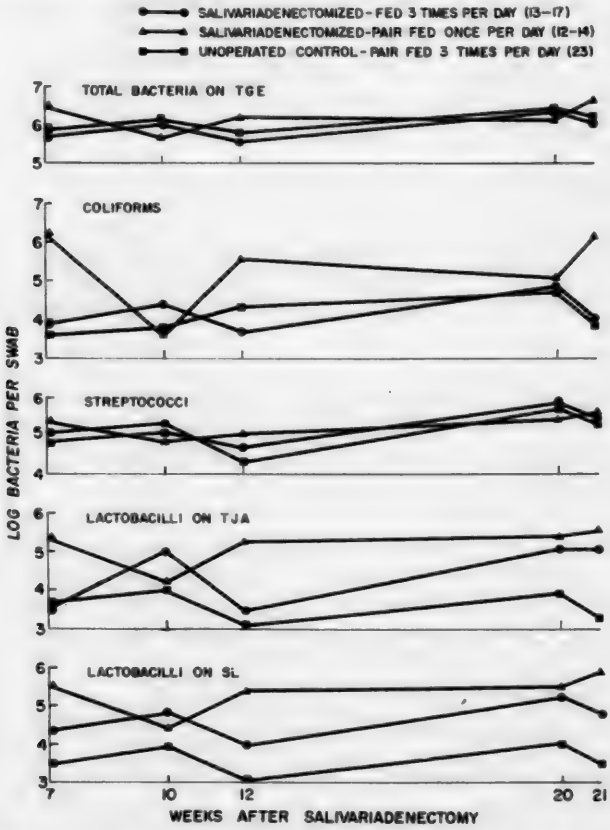

Fig. 1.—Effect of salivariadenectomy on several groups of oral bacteria in caries-resistant rats under controlled feeding conditions. (Numbers in parenthesis indicate the number of rats used.)

The results of the effect of salivariadenectomy on oral bacteria are given in Fig. 1. Lactobacilli were recovered in higher numbers consistently from both groups of salivariadenectomized rats than from unoperated controls when SL agar was used. The numbers of streptococci, coliforms, and total bacteria on TGE agar were not consistently higher in any one group of experimental animals.

The greater numbers of lactobacilli recovered from salivariadenectomized rats than from unoperated controls confirms our previous results.[1] The more rapid development of caries in salivariadenectomized rats than in unoperated controls indicates that lactobacilli may be an etiologic agent for caries in salivariadenectomized rats.

TABLE III

EFFECT OF SALIVARIADENECTOMY ON FOOD AND WATER CONSUMPTION AND WEIGHT, USING CONTROLLED FEEDING CONDITIONS

			40 WEEKS AFTER SALIVARIADENECTOMY				
GROUP	TREATMENT	SEX	NUMBER OF RATS SURVIVING	BODY WEIGHT (GM.)	WEIGHT GAIN (GM.)	FOOD CONSUMED (GM./DAY)	WATER CONSUMED (ML./DAY)
A	Salivariadenectomized (fed 3 times per day)	♂	5	212	39*	9.6	45
A	Salivariadenectomized (fed 3 times per day)	♀	4	199	75	9.1	44
B	Salivariadenectomized (pair-fed to A once per day)	♂	7	253	89	—	44
B	Salivariadenectomized (pair-fed to A once per day)	♀	4	169	51	—	44
C	Unoperated Control (pair-fed to A 3 times per day)	♂	14	312	147	—	27
C	Unoperated Control (pair-fed to A 3 times per day)	♀	9	229	115	—	25

*One of the 5 rats lost 46 grams in weight.

SUMMARY

Rats were fed so that the amount of time spent in feeding, as well as the amount of food consumed, was about the same for salivariadenectomized rats and unoperated controls. The caries time for salivariadenectomized rats was less than that of unoperated controls.

Lactobacilli, but not other groups of microorganisms, were recovered in higher numbers consistently from salivariadenectomized than from unoperated control rats.

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HISTOCHEMICAL ANALYSIS OF LESIONS IN INCIPIENT DENTAL CARIES

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INTRODUCTION

THE pathogenesis of dental caries has been extensively studied in susceptible albino rats. In such animals on cariogenic diets the typical changes associated with dental caries have been detected as early as 48 days of age.[1] From 15 days of age until weaning, these animals had available to them a finely ground polished rice diet which was provided for the mothers. The young were weaned and placed on a coarse polished rice diet at 35 days of age. These have involved actual physical discontinuities in the enamel and dentin, and gross discoloration—presumably all produced through microbial action and accompanied by the presence of microorganisms in the lesions. These changes are considered irreparable in nature and are beyond the recuperative powers of the dental tissues.

It is interesting to suggest, however, that premonitory changes in the dental tissues might precede the classical picture of carious destruction and serve to throw light on underlying mechanisms of pathogenesis of this disease.

With the use of tissue specimens prepared in a manner to minimize postmortem changes, and with the aid of histochemical technics to be described, it has indeed been possible to demonstrate unequivocally subtle but striking functional and structural changes at the site of anticipated gross carious lesions which occur very early after eruption of the teeth.

It is the purpose of this paper, therefore, to describe the innovations in tissue preparation, the application of histochemical methods, and the emerging picture of the premonitory lesion in experimental caries of the albino rat.

MATERIALS AND METHODS

Induction of Caries.—Five hundred caries-susceptible albino rats were utilized in this study. The original stock of Osborne-Mendel strain was obtained from the Naval Medical Research Institute. They were weaned and divided into two groups at 21 days of age. One group was placed on a cariogenic diet of the following composition: casein 24, sucrose 65, corn oil 5, salts U.S.P. XIV 4, dehydrated whole liver 4, vitamin mix 2, choline 0.2 parts. The

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other group of animals were placed on Purina laboratory chow* which is practically noncariogenic. The pellets were ground before feeding. The composition of this ration is given in Table I. With few exceptions, the animals were sacrificed serially from 21 to 50 days of age.

TABLE I

CHEMICAL COMPOSITION OF PURINA CHOW FED TO ANIMALS

CHEMICALS	LABORATORY CHOW
Protein, per cent	25.00
Arginine, per cent	4.30
Glycine, per cent	5.40
Lysine, per cent	5.60
Methionine, per cent	1.70
Tryptophan, per cent	1.30
Cystine, per cent	
Glutamic acid, per cent	16.00
Histidine, per cent	2.40
Leucine, per cent	8 20
Phenylalanine, per cent	4.60
Valine, per cent	5.40
Fat, per cent	6.00
Fiber, per cent	4.50
Ash, per cent	7.00
Nitrogen free extract, per cent	47.50
Calcium, per cent	1.30
Phosphorus, per cent	.90
Potassium, per cent	1.00
Magnesium, per cent	.20
Chlorine, per cent	
Sodium, per cent	.30
Iron, parts per million	325.00
Zinc, parts per million	
Manganese, parts per million	100.00
Copper, parts per million	15.00
Cobalt, parts per million	.10
Iodine, parts per million	2.00
Carotene, parts per million	6 00
Thiamin, parts per million	10.00
Vitamin A, I.U./gram	10.00
Vitamin D, U.S.P. units/gram	5 00
Riboflavin, parts per million	8.00
Niacin, parts per million	100.00
Pantothenic acid, parts per million	15.00
Choline, parts per million	1.700.00
Folic Acid, parts per million	
Pyridoxine, parts per million	4.30
Vitamin C, milligrams/pound (added)	

Preparation of Tissue Sections.—Either thin ground or decalcified sections have usually been employed for the microscopic study of dental tissues. Although these preparations have provided valuable information, they do have certain limitations. In making ground sections, the tubular protoplasm is dehydrated, the pulp is macerated, and the heat generated in the grinding process could inactivate enzymes which might be present.

Serial sections are possible in decalcified material; but the tissue is so altered in the process that it is no longer possible to study the exact conditions

*Purina Laboratory Chow, Ralston Purina Company, St. Louis 2, Mo.

present at the time of death. In both technics, microbial plaques and impacted food particles are usually lost.

The following technic which preserves the food present in the grooves of the teeth, bacterial plaque, enamel, dentin, pulp, and supporting structures was, therefore, employed in this study. The animals were decapitated, the major portion of flesh removed from the jaws and the jaws frozen immediately. The jaws were mounted in ice with the buccal surface horizontal. They were cut with a microtome in a deep freeze. The microtome blade was sharpened similar to a chisel. The cuts were made about 8 μ in thickness and were continued until about one half of the tooth structure was shaved away. The block of ice was then removed with a saw. Another block of ice was shaved until a smooth surface was obtained. The first block of ice was then mounted on this new block with water, the cut surface down. The shaving was carefully continued until sections of from 20 to 30 μ in thickness were obtained. These sections remained frozen until used.

Histochemical Technics.—Decalcifications or other rigorous methods of treatment which would produce postmortem artifacts in the tissues were not employed in this study.

The ninhydrin-Schiff method of Yasuma and Ichikawa[2] was found useful in demonstrating reactive proteins. The Hotchkiss[3] periodic acid method for demonstrating polysaccharides was employed. The method of Macallum[4] was used to demonstrate the presence of sulfur. Microstaining for the presence of reducing agents (e.g., vitamin C) by the method of Eränkö[5] was employed. A modification of the Semenoff[6] technic was used to demonstrate the presence of an oxidation-reduction system. Sections were flooded with 5 per cent methylene blue for 5 minutes, and blotted dry. The sections were then flooded with a solution made up of 2 c.c. of 10 per cent sodium succinated and 6 c.c. of phosphate buffer (0.15 M; pH 7.6). The sections were covered with a cover slip which was sealed with petrolatum. The slides were left at room temperature to incubate and were observed periodically. Decolorization was usually complete in less than 10 hours. When the sodium succinate was omitted no reaction occurred. Likewise, when the sections were exposed to a temperature of 65° C. for 10 minutes, no reaction was observed. Enzyme inhibitions, such as salts of silver, lead, and cyanides, also effectively blocked the reaction.

The following technic was used to demonstrate diffusion through intact enamel. Methylene blue powder was placed in small cloth bags in the mouths of rats at 23, 28, 35, and 45 days of age. The animals were sacrificed after 6 hours. The mandibles were frozen and sectioned as previously described.

Contact microradiographs were made of sections from both groups of animals. A North American Phillips contact microradiograph No. 5, using 1½ Ma. at 5 kv. for 15 minutes, was used. The distance from tube to film was 24 mm.

FINDINGS

Caries-free Rats.—The changes which occur in tooth structure of the Purina-fed animals from 15 to 50 days of age will be considered first. The

technic of Eränkö[5] for reducing agents (e.g., vitamin C) in tissue sections showed histochemical changes in the enamel at various ages. At 15 days of age practically all of the enamel of the unerupted molars stains black. Fig. 1 shows a typical section from a 15-day-old animal. By 19 days of age (Fig. 2), the tips of the cusps no longer stained, while the depth of some of the major grooves stained black and others did not. At 25 days of age, the stained area in the depth of the major grooves appears to coincide with the area of food

Fig. 1.—Sections of 15-day-old animal stained with $AgNO_3$ by the technic of Eränkö. *E*, enamel; *D*, dentin.

Fig. 2.—Sections of 19-day-old animal stained with $AgNO_3$ by the technic of Eränkö. *E*, enamel; *D*, dentin.

impaction and involves the full depth of the enamel. At this age the dentin rarely stains. Fig. 3*A* shows a section of an animal at 25 days of age. At 28 days of age the stained area in the enamel is still present, although not so clearly marked (Fig. 4*A*). Enamel from animals older than 40 days of age no longer exhibited the stain (Fig. 5*A*).

Sections treated for the presence of sulfur showed similar changes in the enamel at the corresponding ages. Since both methods show changes in the same areas it is possible that all of the reactions observed are due to the action of the same reducing agents. The histochemical technics used for the demonstration of carbohydrates and proteins in the sections as prepared showed nothing in the enamel except in lamellae.

In the dentin of sections at all ages treated with $AgNO_3$ a black stain was found in the dentinal tubules. The presence of protein and carbohydrate was also demonstrated in the dentinal tubules by the technics used (Figs. 9*A* and 10*A*). No reactions were observed in the dentinal matrix of any section.

The presence of an oxidation-reduction system was found uniformly throughout the dentin of these animals at all ages studied (Fig. 6, *A*).

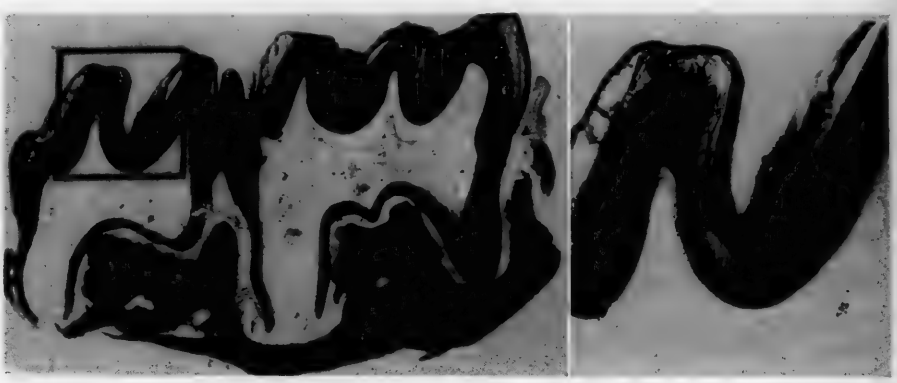


Fig. 3*A*.—Section of 25-day-old animal on noncariogenic diet stained by method of Eränkö. Arrows point to stained area in depth of major groove. *D*, dentin; *E*, enamel.

Fig. 3*B*.—Section of 25-day-old animal on cariogenic diet stained by method of Eränkö. Arrows point to stained area in depth of major groove. *D*, dentin; *E*, enamel; *F*, food.

Contact microradiographs of animals at the various ages studied also showed a uniform density throughout the dentin. Fig. 7, *A* shows a typical microradiograph.

Optically the tubules appeared normal throughout the dentin from these animals (Fig. 8, *A*).

The diffusion of the methylene blue through in vivo tooth structure was largely confined to the enamel and dentin in the depth of the major grooves.

As the animals became older the evidence of diffusion decreased. The cusp tip which is denuded of enamel showed practically no diffusion.

Caries Involved Rats.—Sections of the teeth of animals placed on the cariogenic diet showed the following changes. The enamel of 25-day-old

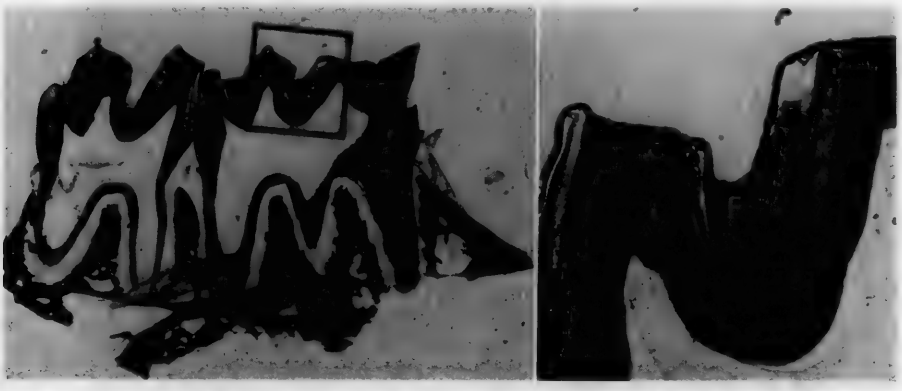


Fig. 4*A*.—Sections of 28-day-old animal on noncariogenic diet stained by the method of Eränkö. Note enamel stained in depth of major groove. *E*, enamel; *D*, dentin; *F*, food.

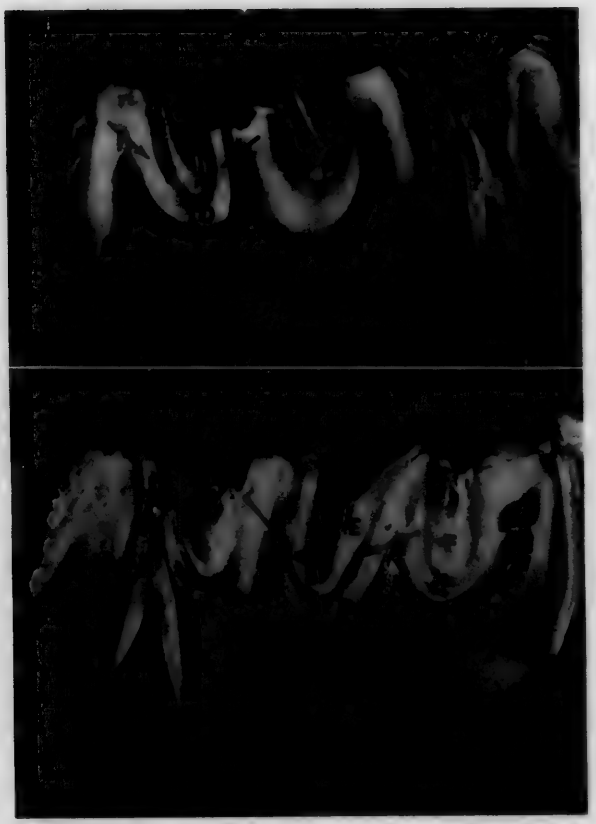
Fig. 4*B*.—Section of 28-day-old animal on cariogenic diet stained by the method of Eränkö. Note stain in enamel and dentin in depth of major groove. *E*, enamel; *D*, dentin; *F*, food.

animals stained intensely in the depth of the major grooves when treated with $AgNO_3$ (Fig. 3*B*). As the animals became older, the enamel continued to stain intensely adjacent to the food in the depth of the major grooves (Figs. 4*B* and 5*B*).

Sections treated for the presence of sulfur showed similar changes to those noted when the sections were treated with $AgNO_3$.

Fig. 5*A*.—Sections of 44-day-old animals on noncariogenic diet stained by method of Eränkö. Arrows point to area in depth of major groove which did not receive the stain. *E*, enamel; *D*, dentin; *F*, food.

Fig. 5*B*.—Sections of 39-day-old animal on cariogenic diet stained by the method of Eränkö. Arrows point to area in depth of major groove which stained deeply. *E*, enamel; *D*, dentin; *F*, food.

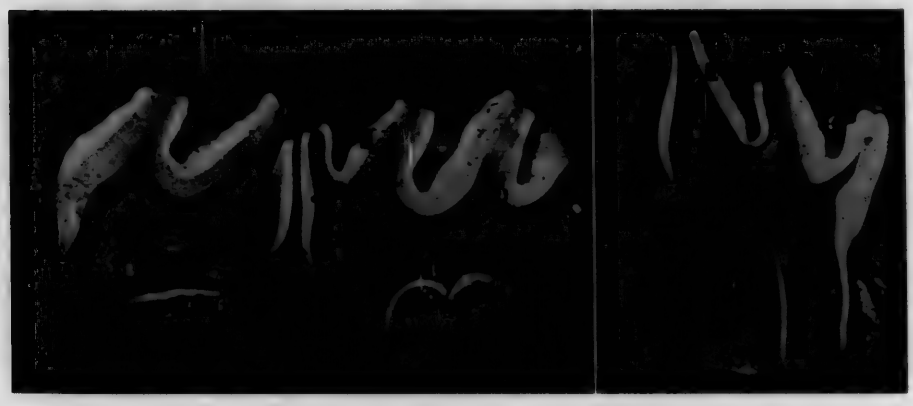


A.

B.

Fig. 6.—*A*, Section of 28-day-old animal on noncariogenic diet treated by technic of Semenoff to show the presence of succinic dehydrogenase. Note uniform decolorization of dentin. *E*, enamel; *D*, dentin; *F*, food.

B, Section of 28-day-old animal on cariogenic diet treated by technic of Semenoff to show presence of succinic dehydrogenase. Note area of incomplete decolorization in dentin. *E*, enamel; *D*, dentin; *F*, food.



A. *B.*

Fig. 7.—*A*, Contact microradiograph of 28-day-old animal on noncariogenic diet. Note uniform density of dentin. *E*, enamel; *D*, dentin.

B, Contact microradiograph of 28-day-old animal on cariogenic diet. Note radiolucent area in dentin. *E*, enamel; *D*, dentin.

The protein and carbohydrate of enamel were unreactive except in the lamellae.

The dentin of animals on the cariogenic diet at 25 days of age stained similarly to the caries-free animals (Fig. 3*B*). The technic of Eränkö[5] showed the presence of reducing substances in the tubules and in the immature dentin adjacent to the pulp cavity. The presence of protein and carbohydrate was observed within the dentinal tubules as before. Figs. 9*B* and 10*B* show sections from animals 28 days of age stained for protein and carbohydrate.

A. *B.*

Fig. 8.—*A*, unstained section of 28-day-old animal. Arrows point to area of change in depth of major groove with normal tubules. *E*, enamel; *D*, dentin; *F*, food.

B, Unstained section of 28-day-old animal. Arrows point to area of change in depth of major groove. Note abnormal appearance of tubules beneath enamel. *E*, enamel; *D*, dentin.

Sections of animals 28 days of age on the cariogenic diet showed marked changes in the dentin in the depth of some of the major grooves. These changes are confined to the area just beneath the enamel adjacent to the impacted food. The dentin in this area stained black by the technic of Eränkö[5] and also by the technic of Macallum.[4] Fig. 4*B* (lower picture) shows a typical section of a 28-day-old animal stained by the technic of Eränkö.[5] The dentinal matrix in this area showed a marked increase in stainable protein (Fig. 9*B*) and a marked increase in stainable carbohydrates (Fig. 10*B*). As the animals became older this area progressed toward the pulp but did not seem to extend any further along the dentinoenamel junction. Fig. 11 shows this in older animals.

The presence of an oxidation-reduction system was found uniformly throughout the dentin except in the dentin in the depth of some of the major grooves (Fig. 6, *B*).

Contact microradiographs of sections from these animals which had been on the cariogenic diet showed that this area of dentin in the depth of some of the major grooves is more radiolucent than adjacent areas in the same tooth (Fig. 7, *B*).

Fig. 9*A*.—Section of 28-day-old animal on noncariogenic diet stained for presence of protein. Note absence of change in depth of major groove. *E*, enamel; *D*, dentin; *F*, food.

Fig. 9*B*.—Section of 28-day-old animal on cariogenic diet stained for presence of protein. Arrows point to area in depth of major groove which stained deeply. *E*, enamel; *D*, dentin; *F*, food.

Optically the tubules in the area of radiolucent dentin appear different from those areas in the same section which are not radiolucent (Figs. 8, *A* and *B*). Apparently the protoplasm within the tubules is affected. A further study of these tubules has been made with the electron microscope confirming these findings and will be reported later.

The diffusion through the enamel and dentin of the animals on the cariogenic diet at the younger age was very similar to that found in the animals on Purina chow. The diffusion was largely confined to the enamel and dentin in the depth of the major grooves. As the animals became older, the areas in

Fig. 10*A*.—Section of 28-day-old animal on noncariogenic diet stained for presence of carbohydrate. Note absence of change in depth of major groove. *E*, enamel; *D*, dentin; *F*, food.

Fig. 10*B*.—Section of 28-day-old animal on cariogenic diet stained for presence of carbohydrate. Note area stained deeply in depth of major groove. *E*, enamel; *D*, dentin.

Fig. 11.—Section of 70-day-old animal on cariogenic diet stained by the technic of Erränkö. Note depth of stained area. *E*, enamel; *D*, dentin.

the depth of some of the major grooves continued to stain darkly in both the enamel and dentin. The general pattern of this area of diffusion as similar to that found with the other technics.

Table II is presented to summarize the histochemical findings of sections from animals of various ages which were on two widely differing dietary regimes. These animals were weaned at 21 days of age. After one week, from 28 days of age and onward, marked differences were found between the two groups of animals in both the enamel and dentin.

TABLE II

RESULTS AT SITE OF CARIES PREDILECTION COMPARED WITH OTHER AREAS IN SAME SECTIONS

	ENAMEL			DENTINAL MATRIX		
	21-27	28-34	35-42	21-27	28-34	35-42
			Caries Free			
Protein	0	0	0	0-×	×-0	0
Carbohydrate	0	0	0	0-×	×-0	0
Silver reducing substance	××	×	0	0-×	×-0	0
Redox activity (succinic (dehydrogenase)	0	0	0	×	×	×
Radiograph		Not interpreted		0	0	0
Diffusion	××	×	×-0	××	××-×	×-0
			Caries Involved			
Protein	0	0	0	0-×	××	××
Carbohydrate	0	0	0	0-×	××	××
Silver reducing substance	××	××	××	0-×	××	××
Redox activity (succinic dehydrogenase)	0	0	0	×	0	0
Radiograph		Not interpreted		0	×	××
Diffusion	××	××	××	××	××	××

Legend: Differences between groups boxed in. 0 = No change. × = Slight change. ×× = Marked change.

DISCUSSION

Fig. 12 is presented to facilitate the discussion. It will be noted that the areas involved in the histochemical changes are outlined.

At 28 days of age, the sections of animals on the cariogenic diet showed marked changes in area 3 of Fig. 12. In this area, a marked increase in stainable protein, carbohydrate, and sulfur was found. There was, also, a loss of mineral content and a cessation of oxidation-reduction.

1. The lesion is characterized by: (a) Persistence of local enamel permeability to methylene blue in Area 2 beyond normal age of maturation. This is not restricted to lamellae but is along a broad front, which includes all of Area 2. (b) Diminished oxidation-reduction activity in Area 3. (c) Loss of mineral phase in Area 3. (d) Increased availability of carbohydrate and protein in Area 3—possibly from mucopolysaccharide of ground substance.[7]

2. Whether these observed changes occur simultaneously or follow some pathogenetic sequence is as yet unknown.

3. To what extent they represent exogenous stimuli of bacterial origin and endogenous impairment is also not clear.

4. Observations made in this study are consistent with the suggestion that the increased availability of carbohydrate and protein is the result of mineral loss.

5. This does not rule out the possible coincident action of a mucopolysaccharide hydrolyzing enzyme capable of inducing the observed changes.

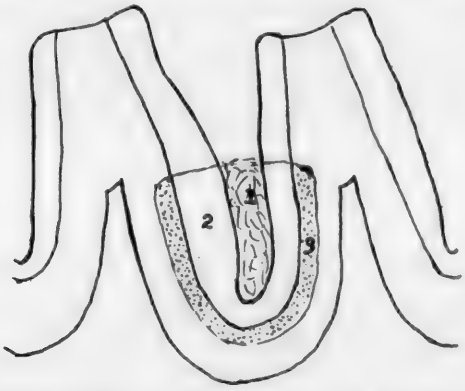


Fig. 12.—Drawing of typical section of a rat molar. *1*, food; in groove; *2*, enamel; *3*, dentin just beneath enamel.

Mucopolysaccharides have been identified by other workers[7] in the organic portion of the tooth. Crude toxins have been isolated from bacteria[8] normally inhabiting the carious lesion. These toxins were found to be capable of hydrolyzing mucopolysaccharides. Diffusable food substances of cariogenic diet may account for reactive protein and carbohydrate in dentin of Area 3, but are hardly responsible per se for changes in oxidation-reduction potential or loss of mineral.

6. The loss of mineral may be the result of primary microbial agencies.

7. It may, also, be the secondary consequence of some induced endogenous cellular impairment suggested by (a) diminished local oxidation-reduction activity, and (b) structural differences in tubular terminal endings.

Further explanation for the character of the premonitory lesion herein described must await further experimental study.

CONCLUSION

Suitable histochemical technics applied to properly prepared tissues reveal striking changes which precede the classical picture of the carious lesion.

These changes include a cessation of oxidation-reduction, an increased stainability of carbohydrate and protein, the presence of a reducing agent, and loss of mineral.

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THE EFFECT OF VARYING PEROXIDE AND POLYMER ON THE RATE AND DEGREE OF POLYMERIZATION OF POLYMETHYL METHACRYLATE SLURRIES

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THIS paper is a continuation of a project described previously.[1] A number of new promoter systems have been suggested for use in self-curing dental plastics in the last several years. Many of the fundamental relationships can be investigated by studying variables in a well-known powder-liquid system: a mixture is used consisting of two parts by weight of a methyl methacrylate polymer or copolymer and one part by weight of a methyl methacrylate monomer. Benzoyl peroxide is used as the catalyst and N,N-dimethyl-p-toluidine is the promoter.

In the previous paper, rates of reaction were measured by time of heat peak (the maximum temperature reached by an equilibrium of heat created by exothermic polymerization and the cooling due to heat conduction or radiation.)[2, 3, 4] At the time of heat peak these slurries are clinically hard. The temperature increase was measured. With the system studied, it was found to be inversely proportional to the time of the heat peak. The properties of various promoters were also compared.[1] It was discovered that the heat peak temperatures and times fit into a family of curves which could be superimposed by varying the concentrations of the amine or the catalyst used. Certain catalysts appear to be more efficient and to give a more rapid heat peak than others, and similarly various amines, such as aromatic dimethyl-p-toluidine, appear to be more efficient than an aliphatic amine such as trihexylamine.

In this paper we are interested in what happens beyond the heat peak. What is the effect of various catalysts, having the same heat peak time and temperature peak, on the rate and degree of polymerization after the time of the heat peak?[5] This is of practical importance to the dentist who is interested in a filling or denture having minimum distortion or residual monomer at the time of insertion.

Variations within one catalyst-promoter system generally follow a uniform pattern. For benzoyl peroxide and dimethyl-p-toluidine, the more the catalyst and promoter, up to a useful maximum concentration, the more rapid is the polymerization. This is indicated by a faster heat peak with a higher temperature and more complete polymerization. Whether this relationship holds true for different promoter systems is the subject of this paper.

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EXPERIMENTAL PROCEDURE

This study was conducted to establish data on speed of polymerization in autopolymerized methyl methacrylate slurries in relation to the degree of polymerization at various times after the heat peak time. To determine heat peak time, a procedure was followed similar to that used by Walcott, Paffenbarger, and Schoonover.[2] The degree of polymerization was measured by use of a disk of polymer, 3 mm. thick by 39 mm. in diameter, prepared in a stainless steel mold. Our measurement of comparative completion of polymerization was made by using a Rockwell indentation test which can be correlated with residual monomer.

Graph 1 is a comparison of Rockwell M and Residual Monomer. These results are roughly proportional, but will vary, depending on the components. Steck[6] tested residual monomer in a promoted system and found that the curing continued up to 500 hours and was a curve of the same shape as our Rockwell curve starting at 8 per cent residual monomer in one hour and down to 4.5 per cent at the end of the test (*Graph 2*). A Knoop-Peters indenter, Vickers, or any other indentation tester can be used similarly as long as it will differentiate properties of hardness or indentation which can be correlated with percentages of monomer and can be calibrated. Residual monomer can be determined in any of the samples by titration.[7, 8] Although the size of the sample is important in the degree of polymerization obtained, a comparative degree of polymerization among different systems can be determined by comparing identically sized samples.

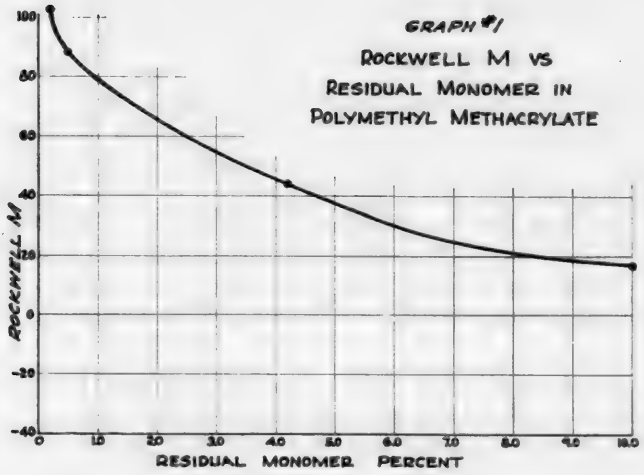
Surface inhibition of polymerization by air or moisture cannot be quantitatively determined easily. However, visual evidence of a soft film on the sample surface would indicate inhibition. This air inhibition would affect a Rockwell indentation determination more than a residual monomer determination, since the residual monomer would measure bulk properties and not surface effect. To study this property, a Rockwell indentation determined on an exposed surface can be compared with a Rockwell indentation on a surface protected from the atmosphere so that a qualitative comparison of surface inhibition can be made. In all the experiments described, the surfaces were polymerized under pressure against "Mylar" and inhibition was minimized.

In *Graph 3*, with constant percentage of amine, benzoyl peroxide is compared with p-chlorobenzoyl peroxide in time to peak temperature. It is to be noted that the p-chloro form is more rapid than the benzoyl peroxide.

Similarly, with the same amount of peroxides the percentage of amine is changed and the results are compared in *Graphs 4* and *5*. Note that the p-chlorobenzoyl peroxide is more rapid than benzoyl peroxide when the same percentage is used. The molecular weight is 30 per cent higher, giving an even greater reactivity to the p-chlorobenzoyl peroxide. This enhanced reactivity can be explained on the basis of the electron withdrawing character of the chlorine group, which makes p-chlorobenzoyl peroxide more stable to heat, but less stable in the presence of monomer and amine.[8]

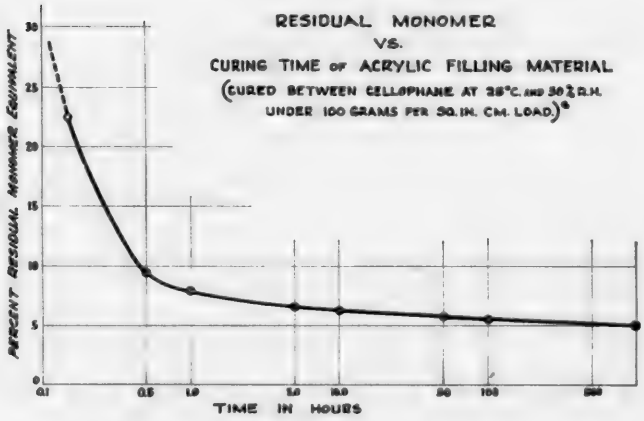
GRAPH #1

ROCKWELL M VS
RESIDUAL MONOMER IN
POLYMETHYL METHACRYLATE



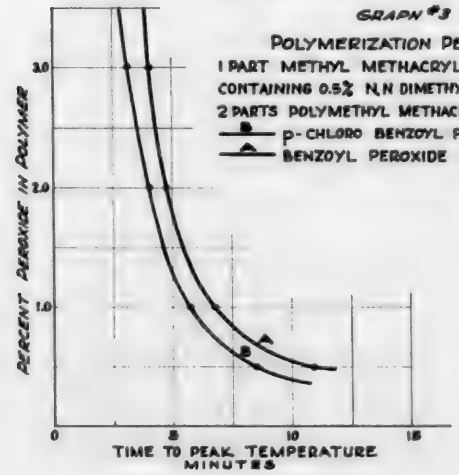
GRAPH #2

RESIDUAL MONOMER
VS.
CURING TIME OF ACRYLIC FILLING MATERIAL
(CURED BETWEEN CELLOPHANE AT 28°C. AND 50% R.H.
UNDER 100 GRAMS PER SQ. IN. CM. LOAD)



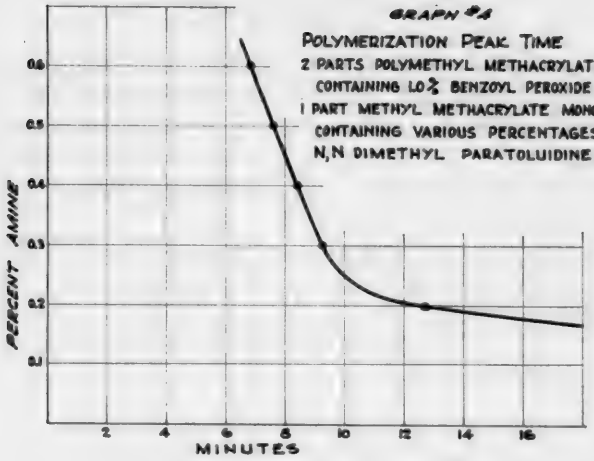
GRAPH #3

POLYMERIZATION PEAK TIME
1 PART METHYL METHACRYLATE MONOMER
CONTAINING 0.5% N,N DIMETHYL p-TOLUIDINE.
2 PARTS POLYMETHYL METHACRYLATE CONTAINING
B p-CHLORO BENZOYL PEROXIDE IN POLYMER.
A BENZOYL PEROXIDE IN POLYMER.



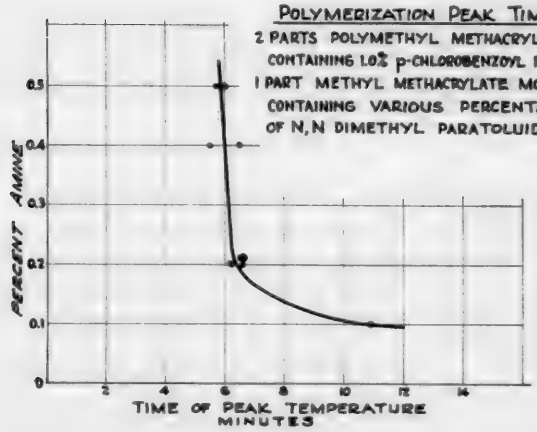
GRAPH #4

POLYMERIZATION PEAK TIME
2 PARTS POLYMETHYL METHACRYLATE CONTAINING 1.0% BENZOYL PEROXIDE AND 1 PART METHYL METHACRYLATE MONOMER CONTAINING VARIOUS PERCENTAGES OF N,N DIMETHYL PARATOLUIDINE.

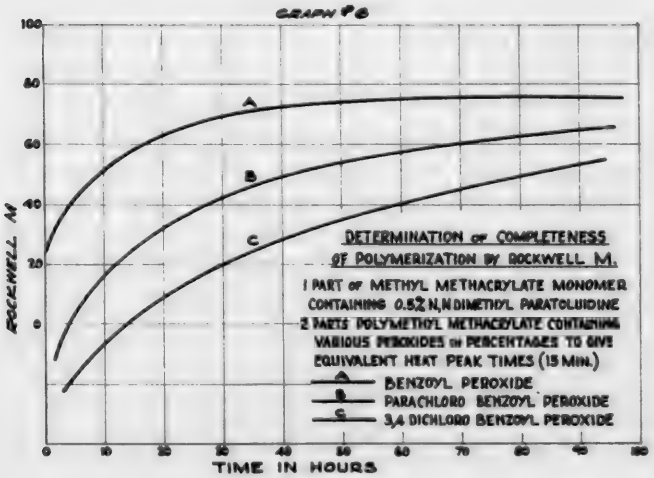


GRAPH #5

POLYMERIZATION PEAK TIME
2 PARTS POLYMETHYL METHACRYLATE CONTAINING 1.0% p-CHLOROBENZOYL PEROXIDE AND 1 PART METHYL METHACRYLATE MONOMER CONTAINING VARIOUS PERCENTAGES OF N,N DIMETHYL PARATOLUIDINE.



GRAPH #6



In *Graph 6,* the completeness of cure is shown for various types of peroxides by plotting the Rockwell M indentations against time. The polymerization rate as measured by heat peak time was identical for all three of these systems. Dimethyl-p-toluidine, 0.5 per cent, was used in the monomer of the dough mix with these compositions. This graph points out that equivalent times of surge or peak temperature do not necessarily mean that the completion of cure is identical or even similar. There does appear to be a consistent pattern when different concentrations are used in one promoter system. Different catalysts and promoters need not show equivalent curing rates or degree of polymerization even though the original heat peak and temperatures were the same.

CONCLUSIONS

1. It is apparent that with different promoter systems there is not always a linear relationship between heat peak time and rate of completion of polymerization beyond the heat peak.[5]

2. Although the polymerization of some systems such as p-chlorobenzoyl peroxide and dimethyl-p-toluidine is more rapid initially than systems employing benzoyl peroxide and dimethyl-p-toluidine, it is slower beyond the heat peak.

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A SURVEY OF LACTOBACILLI INCLUDING PIGMENTED STRAINS FROM THE ORAL CAVITY OF THE WHITE RAT

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DESPITE the routine use of rats in experimental dental caries research, detailed information on the oral bacteriology of these animals is lacking. Although a number of reports have appeared on the acidogenic flora of the rat,[1-8] few are concerned with the nutrition and metabolism of specific organisms.[9, 10] It is the purpose of this paper to present biochemical data on lactobacilli isolated from the rat on a selective medium and to discuss the nutrition and classification of these organisms. Pigmented heterofermentative strains, apparently indigenous to the rat, are also described.

MATERIALS AND METHODS

A total of 132 Sprague-Dawley female rats from the NIH breeding colony were sampled. Sixty were weanling rats about 21 days of age. While suckling they also had access to the Purina rat chow provided for the mothers. Seventy-two of the animals were mature (110 days of age). Of these, 36 were being fed a high sugar cariogenic diet (585)[6] and 36 were on a noncariogenic diet (550).[11]

The animals were distributed in groups of 3 in screen-bottom cages. All food was removed from the cages on the evening of the day before sampling. The molar tooth and gum areas were swabbed and the samples diluted as described by Stephan and his co-workers.[6] Pour plates of Rogosa's selective lactobacillus medium (SL),[12] containing 0.5 per cent each of arabinose and sucrose and 1 per cent dextrose, were incubated aerobically for 4 days at 37° C.

Representative colonies were picked into stabs of the modified SL medium described later. No attempt was made to obtain a statistical sample of the colonies appearing on the plates but an effort was made to obtain representatives of each colonial type which appeared. A total of 77 strains from weanling rats and 85 strains from adult rats were selected for detailed study. All satisfied the following requirements for inclusion in the genus Lactobacillus: gram-positive nonmotile, nonsporulating, and unbranched rods which did not produce catalase, liquefy gelatin, or reduce nitrate to nitrite. Heterofermentation, as evidenced by gas production, was detected by inoculation into agar stab tubes sealed with

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a petroleum jelly-paraffin mixture and by the use of Durham tubes in a broth medium. Comparable results were obtained. Unless otherwise specified, the basal medium employed for classification tests was a modification of the SL isolation medium[12] in which glacial acetic acid and agar were omitted and the pH was adjusted to 6.5, using 10 per cent NaOH. Incubation was always at 37° C. unless otherwise indicated.

Fermentations of individual carbohydrates were detected, using glucose-free basal medium with 0.0016 per cent bromcresol purple as an indicator and one per cent of the desired carbohydrate. Sterilization was by filtration.

Representatives of each biochemical group were tested for type of lactic acid produced, using essentially the method of Curran, Rogers, and Whittier.[13]

Determinations of optimum temperature for growth were based on rate, as well as amount, of growth at 20° C., 25° C., 30° C., 37° C., and 45° C. in a broth medium.

The nutritional medium, described by Rogosa and his colleagues,[14] was employed for the determination of vitamin requirements. In these and other tests the inoculum was one drop from an 18- to 24-hour broth culture which had been washed twice and diluted 1:200, using sterile distilled water.

RESULTS AND DISCUSSION

It was previously reported that the ratio of heterofermentative to homofermentative lactobacilli may vary depending on the age of the rat or the type of diet.[15] Differentiation between the two types could be accomplished on the basis of appearance of subsurface colonies in SL medium as observed under a dissecting microscope. Heterofermentative strains characteristically produced white or pigmented, smooth lens-shaped homogeneous colonies, a millimeter or more in diameter. Colonies of homofermentative strains, on the other hand, ranged in size from 1 mm. down to pinpoint size. Most were gray or waxy and had a curdled, heterogeneous appearance. These observations were based on the isolation and testing of hundreds of colonies. Further correlation between colonial morphology and individual species, described in this paper, was not readily apparent.

Heterofermentative Lactobacilli.—All of the heterofermentative strains isolated from the rat oral cavity could be considered variants of *Lactobacillus fermenti.* This has been reported as the predominant heterofermentative type in surveys of human oral lactobacilli[14, 16] and found in the cecal feces of turkeys by Harrison and Hansen.[17] The strains isolated in the present study were typical in that they produced inactive lactic acid, grew better at 45° C. than at 37° C. or below, did not hydrolyze hippurate, or require extrinsic folic acid.[14] Some strains fermented arabinose or xylose separately, but none fermented both pentoses.

Some variation was seen in the fermentation of mannose by the strains from adult rats which is in accord with observations on the fermentation of this sugar by *L. fermenti* from human beings.[14]

In his original description, Orla-Jensen[18] stated that *Betabacterium longum (L. fermenti)* does not attack arabinose but may attack xylose. Arabinose-fermenting strains of *L. fermenti* have been described from human beings.[14, 16] Apparently, it is not uncommon for variants of this species to ferment either pentose separately. The opinion is stated in Bergey's manual[19] that *L. fermenti* seldom ferments pentoses but, if this fermentation takes place, it is not as active as that of *L. brevis*. The arabinose-fermenting strains from the rat which are described here are otherwise identical to easily recognized *L. fermenti* strains.

Rosen and co-workers[7] did not report the presence of heterofermentative lactobacilli in a bacteriologic study of rats. The reasons for this are not known, although differences in the isolation medium used, as well as the diet of the animals, could be contributory. In our experience, supplementation of the SL medium with arabinose and sucrose makes it more suitable for the isolation of rat lactobacilli since both heterofermentative and homofermentative strains have been encountered which did not ferment glucose on initial isolation.

TABLE I

CHARACTERISTICS OF HETEROFERMENTATIVE LACTOBACILLI FROM RATS

	LACTOBACILLUS GROUP FROM WEANLING RATS				LACTOBACILLUS GROUP FROM ADULT RATS			
	I	II	III	IV	V	VI	VII	VIII
No. of strains	15	6	17	11	4	9	7	4
Arabinose	15+	0	17+	0	0	9+	7+	4+
Mannose	0	0	0	0	0	0	7+	4+
Xylose	0	0	0	0	4+	0	0	0
Levulose	0	0	0	0	0	5+	4+	0
Esculin	0	0	7+	0	0	9+	7+	0
α-Methyl glucoside	12+	6+	17+	11+	0	3+	2+	0
Optimum temperature, degrees C.	40-45	40-45	40-45	40-45	30-37	40-45	40-45	40-45
Pigment	15+	6+	0	0	0	0	0	0

All ferment: glucose, melibiose, lactose, raffinose, sucrose, maltose, galactose.
Do not ferment: mannitol, cellobiose, trehalose, sorbitol, rhamnose, melezitose, sorbose, glycerol, salicin (seven strains in groups VI and VII weakly fermented this sugar).
Other characteristics: inactive lactic acid produced, hippurate not hydrolyzed, NH_3 produced, folic acid not required, thiamin and niacin required.

Pigmented Lactobacilli.—Variant groups I and II from weanling rats (Table I) are composed of pigmented strains which exhibit a rusty-yellow or orange-colored growth. Although not shown here, these strains can be isolated from older rats, usually with less frequency. Breed and Pederson[20] have described pigmented varieties of *L. brevis* and *L. plantarum* from "rusty spot disease" of cheddar cheese. Strains from this source have been studied in more detail by others.[21-25] Tilden and Svec,[16] in their survey of lactobacilli from children, mentioned that older cultures of certain *L. fermenti* strains displayed a yellow pigmentation.

Amino acid requirements of a single pigmented strain (NR 77) were determined in the synthetic medium described by Dunn and his co-workers[26] and were essentially in agreement with requirements listed by these authors for

L. fermenti. Valine, threonine, isoleucine, glutamic acid, tyrosine, serine, aspartic acid, arginine, leucine, alanine, and tryptophane were required. Hydroxyproline, proline, glycine, lysine, cysteine, and histidine were not required. Omission of phenylalanine and methionine resulted in only partial growth and no pigmentation. In the tubes from which cysteine was omitted, pigmentation was markedly delayed even though growth was not restricted. The reducing properties of this amino acid may be necessary for early pigment production.[24, 25] Further studies on the biogenesis of pigment by this organism are in progress.

Homofermentative Lactobacilli.—Several species of homofermentative lactobacilli appear indigenous to the oral flora of the rat. In the present study there were some differences in the occurrence of individual homofermentative groups relatable to the age of the animals, but none which could be attributed to the different diets of the adult rats. Consequently, in the summation of the findings, no distinction is made between the two adult groups on the cariogenic or noncariogenic diet.

Lactobacillus acidophilus: The four biochemical groups shown in Table II appear to be variants of *L. acidophilus.* These strains produced inactive lactic acid, did not hydrolyze hippurate or α-methyl glucoside, and did not ferment mannitol. Best growth was obtained at 37° C. The unstable fermentative character of *L. acidophilus* was evidenced by many slow or weak reactions to certain sugars, notably cellobiose, levulose, salicin, mannose, and melibiose. Vitamin requirements could not be determined with any degree of certainty. Repeated testing gave conflicting results, due to the unsuitability of the nutritional medium for optimal growth of these strains. Folic acid and niacin were probably required and riboflavin appeared to be a requirement of many strains. It is of interest that only four strains (5 per cent) from weanling rats appeared to be variants of *L. acidophilus,* while 23 per cent of the isolates from adult rats apparently belong to this species.

The strains classified as *L. acidophilus* in this study conformed to descriptions given for this species by Curran, Rogers, and Whittier,[13] Rogosa and his co-workers,[14] and in Bergey's manual.[19]

TABLE II

CHARACTERISTICS OF *L. acidophilus* VARIANTS FROM RATS

	LACTOBACILLUS GROUP FROM ADULT RATS			LACTOBACILLUS GROUP FROM WEANLING RATS
	I	II	III	IV
No. of strains	7	14	3	4
Mannose	0	14+ (weak)	3+	4+
Trehalose	7+	14+	0	3+
Lactose	3+	7+	3+	3+
Raffinose	0	3+	1+	2+
Melibiose	0	6+ (weak)	3+	1+
Esculin	0	0	0	3+

All ferment: glucose, sucrose, galactose, cellobiose (slow), levulose (slow), salicin (weak).

Do not ferment: arabinose, mannitol, xylose, rhamnose, melezitose, sorbitol, sorbose, glycerol.

Other characteristics: optimum temperature 37° C., inactive lactic acid produced, hippurate and α-methyl glucoside not hydrolyzed.

Lactobacillus plantarum: Two well-defined groups of homofermentative lactobacilli differing from each other, primarily in the fermentation of mannitol, were isolated from weanling rats. These strains showed a marked similarity to lactobacilli isolated from the cecal feces of turkeys by Harrison and Hansen,[17] and classified by these workers as *L. plantarum.* All strains fermented melibiose which is one of the major differences between this species and *L. casei.* In contrast to the prevalence of *L. casei* in human oral samplings,[14, 16] none of the strains isolated from the rat in this study could be classified as *L. casei.* This species was also not found in a survey of lactobacilli from the hamster by other workers.[27]

Characteristics of the two *L. plantarum* variants from weanling rats are shown in Table III.

TABLE III

CHARACTERISTICS OF *L. plantarum* VARIANTS FROM WEANLING RATS

	LACTOBACILLUS GROUP	
	I	II
No. of strains	9	15
Mannitol	9+	0
Esculin	7+	13+

All ferment: glucose, sucrose, galactose, cellobiose, levulose, salicin, maltose, mannose, melibiose, raffinose, lactose.

Do not ferment: arabinose, xylose, rhamnose, melezitose, sorbitol, sorbose, glycerol, trehalose.

Other characteristics: optimum temperature 37° C., D-lactic acid produced, hippurate and α-methyl glucoside not hydrolyzed.

Other Homofermentative Lactobacilli.—A series of 37 other homofermentative lactobacilli from adult rats were separated into three compact groups based on differences in their action on mannose, trehalose, lactose, hippurate, and esculin. This is shown in Table IV. They are not easily matched with descriptions of known lactobacilli. The melibiose-fermenting groups I and II strains may be varieties of *L. plantarum;* however, they produce a different type of lactic acid than the strains isolated from weanling rats. Actually, in

TABLE IV

CHARACTERISTICS OF HOMOFERMENTATIVE LACTOBACILLI FROM ADULT RATS

	LACTOBACILLUS GROUP		
	I	II	III
No. of strains	17	8	12
Mannose	16+	0	12+
Trehalose	16+	8+	1+
Lactose	16+	0	10+
Salicin	17+	8+	9+
Raffinose	16+	8+	12+
Melibiose	17+	8+	6+
Hippurate	17+	0	0
Esculin	0	7+	12+

All ferment: glucose, sucrose, galactose, cellobiose, levulose, maltose.

Do not ferment: arabinose, xylose, mannitol, rhamnose, melezitose, sorbitol, sorbose, glycerol.

Other characteristics: inactive lactic acid produced, α-methyl glucoside not hydrolyzed.

his original description of this species, Orla-Jensen indicated that optically inactive lactic acid could be produced by this species. There were also sufficient differences in the fermentation of certain sugars to justify separation of these strains from the *L. plantarum* types already described.

The group III strains in Table IV showed more of a tendency toward the characteristics of *L. acidophilus* and actually were quite similar to the group IV strains of *L. acidophilus* from weanling rats described in Table II. However, the group III strains in Table IV were distinctive in their ability to grow well in the nutritional medium and their rapid fermentation of carbohydrates (cellobiose, levulose, salicin) which were slowly or weakly fermented by the weanling rat strains.

An additional group of 15 miscellaneous homofermentative strains from weanling and adult rats were not included in the tabulation. They did not fit into any of the previously described groups nor were they homogeneous within themselves.

SUMMARY

The oral cavity of weanling and adult rats was sampled for lactobacilli, using a selective medium. Variants of *L. fermenti* were the only heterofermentative type found. These included chromogenic strains which produced a rusty-orange pigment.

L. plantarum was the principal homofermentative species found in weanling rats. *L. acidophilus* and three previously undescribed homofermentative lactobacillus types predominated in the adult rat.

Nutritional and biochemical characteristics of the rat oral lactobacilli have been presented.

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RELATIONSHIP BETWEEN MATERNAL INGESTION OF CARBOHYDRATE IN THE RAT AND DENTAL CARIES SUSCEPTIBILITY OF THE PROGENY

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IT HAS become generally accepted that the increased consumption of refined carbohydrates results in a significant increase in the incidence of dental caries in those human subjects who have a specific susceptibility to these food factors. Further support of this relationship is suggested in the data indicating a significant reduction in dental decay in most such people who abstain from the excessive use of refined carbohydrates, especially when taken in between meals. In contrast to these purely local mechanisms of caries initiation, carbohydrates may produce dental caries by a systemic effect. Steinman[1] has shown, for example, that high dietary levels of carbohydrates may affect the calcification of the teeth adversely. Sognnaes[2] has shown in mice, rats, and hamsters that when the pregnant animals receive a 67 per cent carbohydrate diet their progeny have an increased incidence of dental caries.

Calcification is presently thought of as a precipitation of insoluble calcium phosphate salts into an organic matrix, the precipitation of which results when the solubility product of the calcium salts has been exceeded. This occurs when organic phosphate esters are acted upon by the appropriate enzyme systems yielding inorganic phosphate. The organic phosphate esters are hexose or hexose derivatives supplied metabolically in part by dietary carbohydrate. In this way, the level of carbohydrates in the maternal diet may affect the calcification process of the tooth. Whatever the pathway by which dietary carbohydrate acts on calcification, the structure of bone and dental enamel may be adversely influenced by its relative abundance in the diet, especially when it substitutes for other essential food factors. The present study was undertaken to learn more about the mechanism between the maternal ingestion of refined carbohydrates in rats during gestation and lactation and the dental caries susceptibility of the offspring.

EXPERIMENTAL PROCEDURE

A total of 17 female Sprague-Dawley strain rats, approximately 150 days of age, were used for breeding in these studies. All of these animals had consumed a stock corn diet since weaning, and were divided into 2 groups. Group

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A, containing 9 females, was placed on a high carbohydrate diet, while Group B, containing 8 females, remained on the stock corn diet. The composition of these diets are seen in Table I, and their chemical analysis in Table II. The two groups of females were mated with males of the same age and strain. All of the animals were housed, in groups of 2 females and 1 male, in large, raised, screen cages in an air-conditioned room. Feed and fluoride-low distilled water (F = 0.05 μg/ml.) were available ad libitum to all of the parents and their pups.

TABLE I

COMPOSITION OF DIETS USED IN THIS STUDY

DIETARY COMPONENT	DIETS (%) STOCK CORN	HIGH CHO
Yellow corn grits	52.7	
Ground yellow corn	11.3	
Powdered whole milk	30.0	
Alfalfa	4.8	
Iodized sodium chloride	1.0	
Irradiated yeast	0.2	
Sucrose		60
Vitamin test casein		30
Cottonseed oil*		5
Vitamin mixture†		1
Inorganic salts†		4
Percomorph oil		‡

*Wesson.
†See Muhler, J. C.: J. Nutrit. 54: 481, 1954.
‡Fifteen drops per kilogram of diet.

TABLE II

CHEMICAL ANALYSIS OF DIETS USED IN THIS STUDY

DIET	CALCIUM (MG. %)	PHOSPHORUS (MG. %)	FLUORIDE (μG/GM.)	Ca:P
Stock corn	0.37 ± 0.02*	0.32 ± 0.03	1.03 ± 0.05	1.16
High carbohydrate	0.57 ± 0.04	0.51 ± 0.01	1.36 ± 0.06	1.12

*Standard deviation.

At the time the female became pregnant (as determined by body weight gain), the males were removed from the cage and fluoride-free filter paper placed inside with which the mothers could make their nests. Each litter was weaned at 21 days of age, and those in group A were subdivided into two groups. One subgroup, designated group AA, was retained on the high carbohydrate diet, and the other subgroup, designated group AB, was placed on the stock corn diet. The litters born to mothers in group B were similarly subdivided into two groups.

The four groups of offspring were sacrificed at 121 days of age. Immediately before sacrificing, a blood sample was withdrawn by cardiac puncture, and analyzed for calcium,[3] phosphorus,[4] glucose,[5] and chloride.[6] The final body weights were recorded for each group and the heads and left femur were removed from each animal. The dental caries incidence was scored by the method described of Muhler[7] and the ashed femurs were analyzed for calcium and phosphorus. The ash in the fat free femur was determined by ether-alcohol extraction.

DATA AND DISCUSSION

It was extremely difficult to obtain pups from the parents receiving the high sucrose diet (Table III). This has been repeatedly mentioned by Muhler[8] in his studies using a similar diet. Thus, a modification of the high sucrose diet was made which involved the substitution of cottonseed oil for the corn oil. This group was designated group A′. The animals in this group also differed in that the parents were raised on a high sucrose diet in contrast to the animals of group A which had been raised on a stock corn diet. The litters born to group A′ showed a marked increase in the number of animals reaching weaning age, although the explanation for this is not known. At present, it does not seem to be the simple substitution of one form of fat for another. The litters of this group were similarly subdivided and treated exactly as those of groups A and B.

TABLE III

REPRODUCTION COMPARISONS OF FEMALES RECEIVING THE DIFFERENT DIETS

DIET	NO. OF BREEDINGS	NO. OF FEMALES USED	FEMALES BEARING LITTERS	NO. OF LITTERS	PUPS AT BIRTH	PUPS AT WEANING
A = High CHO	16	9	5	8	41	9
B = Stock corn	16	8	7	13	92	54
A′ = modified CHO	5	5	5	5	50	42

The effects of the different dietary regimes on the incidence of dental caries are presented in Table IV. The animals of group A→B (those born to mothers consuming the high sucrose diet and which were placed on a stock corn diet at 21 days of age) had significantly fewer carious lesions than the animals of group B→B (those born on the stock corn diet and which remaining on this diet). In animals of group B→A (those born to mothers consuming the stock corn diet and which were placed on the high carbohydrate diet) an increased number of caries were found when compared to pups on A→A (who were born on the carbohydrate diet and remaining on it). There were no dental caries in any of the animals which were born from parents receiving the high sucrose diets and whose pups remained on it throughout the study (group A→A). These data differ absolutely from those of Sognnaes[2] which suggested that the caries incidence is significantly increased in those progency from parents receiving a high carbohydrate diet.

TABLE IV

THE DENTAL CARIES EXPERIENCE OF RATS BORN FROM MOTHERS INGESTING THE VARIOUS DIETS

DIET	NO. OF RATS	MEAN NO. OF LESIONS	MEAN SEVERITY OF LESIONS	"t"			P		
A remaining on A	4				1.22			0.2	
A placed on B	5	2.2 – 0.6*	1.1 – 0.3			---			
B remaining on B	24	8.9 – 0.6	2.2 – 0.6	---					
B placed on A	25	0.4 – 0.1	0.3 – 0.2	4.8	---		0.001		
A′ remaining on A′	20	2.0 – 0.5	1.0 – 0.4						
A′ placed on B	20	7.8 – 0.4	2.3 – 0.6	0.9	3.6	5.9	0.5	0.001	0.0001

A = high CHO; B = stock corn; A′ = modified high CHO.
*Standard deviations.

The possibility that differences in the caries incidence were the result of differences in the post-weaning diet cannot explain these data. The only apparent difference in experiment design between the study of Sognnaes and this study is in the length of time the female rats received the sucrose diet prior to pregnancy. The animals in the present study were placed on the high carbohydrate diet just before breeding, but in the Sognnaes study the animals were given the carbohydrate diet not only during pregnancy but for "some time" before conception. The exact time was not reported.

Providing this is the only difference between these two studies, it is of interest to examine the data on the litters born to mothers receiving diet A who, in contrast to group A females, had been reared on the high sucrose diet from birth. These data show that there was no significant differences in the dental caries incidence between animals born on a carbohydrate diet (A′→B) and those born on a stock corn diet (B→B). This contrasts sharply with the significant difference seen in the dental caries incidence of pups born to females on the stock corn diet, compared to those born to parents receiving the high carbohydrate diet only during pregnancy and lactation (A→B). It is seen that the animals born of mothers which had received the high sucrose diet from the time they were born and then the progeny remained on this high carbohydrate diet had a mean number of lesions of 2 (A′→A′), while similar mothers which received the high carbohydrate diet only during pregnancy and lactation and whose progeny remained on the high carbohydrate diet had no lesions (A→A). The influence of the *Mother's diet prior to pregnancy* on the caries susceptibility of the offspring is a factor heretofore not recognized, and obviously needs much further investigation.

A number of chemical determinations were performed in an attempt to discover differences in the blood, saliva, or teeth themselves, other than the dental caries incidence, which might indicate the reason for the differences observed in the various groups.

Whole tooth intact enamel solubility determinations were performed on 36-day-old rats of the various groups by methods previously described by Buttner and Muhler.[9] These data (Table V) indicate that those teeth which calcified while receiving the high carbohydrate diet had enamel which was less soluble than the enamel of animals born on the stock diet. However, these differences had only a borderline significance ($p = 0.06$). Nevertheless, it is interesting that the tendency toward decreased enamel solubility is in the same direction as the dental caries incidence.

TABLE V

ENAMEL SOLUBILITY OF 30-DAY-OLD RAT MOLARS FROM ANIMALS BORN ON THE DIFFERENT DIETS

DIET	PHOSPHORUS REMOVED (μG P)
Born on stock corn	62 ± 3*
Born on High CHO	55 ± 2

*Standard deviation.

Calcium and phosphorus analysis of femurs similarly revealed no differences between any of the groups. The ash content in the fat free femur was remarkably constant among the different groups. These data are seen in Table VI. Blood analysis for calcium, phosphorus, glucose, and chloride revealed no significant differences in the levels of any of these constituents among any of the groups (Table VII).

TABLE VI

FEMUR ANALYSIS AND BODY WEIGHT OF RATS RECEIVING DIFFERENT DIETS

DIET	FINAL BODY WEIGHT (GM.)	% ASH IN FAT FREE FEMUR	Ca (MG. %)	P (MG. %)
A remaining on A	227	63.87 ± 0.86*	34.5 ± 1.1	16.4 ± 0.3
A placed on B	255	65.78 ± 0.46	35.6 ± 1.2	17.6 ± 0.5
B remaining on B	252	64.66 ± 0.42	34.8 ± 1.0	17.8 ± 0.7
B placed on A	211	63.59 ± 1.16	34.2 ± 0.9	15.4 ± 0.4
A′ remaining on A′	244	64.30 ± 0.35	35.1 ± 2.1	15.7 ± 0.2
A′ placed on B	252	66.52 ± 0.60	35.4 ± 1.8	17.5 ± 0.6

*Standard deviation.

TABLE VII

BLOOD ANALYSIS FOR CALCIUM, PHOSPHORUS, GLUCOSE, AND CHLORIDE IN RATS RECEIVING DIFFERENT DIETS

	SERUM CALCIUM (MG. %)	SERUM PHOSPHORUS (MG. %)	BLOOD GLUCOSE (MG. %)	CHLORIDE (mEq./ML.)
A→A	8.5 ± 0.6*	4.8 ± 0.4	106 ± 7	84.7 ± 1.8
A→B	8.0 ± 0.5	4.6 ± 0.3	105 ± 5	89.3 ± 2.2
B→B	8.6 ± 0.3	4.5 ± 0.3	112 ± 6	88.8 ± 1.2
B→A	8.5 ± 0.4	4.4 ± 0.2	109 ± 6	87.1 ± 2.0

*Standard deviation.

Salivary flow has been suggested by many workers to influence the dental caries incidence. Consequently it was considered of value to investigate these factors in an attempt to explain the differences in dental caries. The data indicate (Table VIII) that animals born on the high carbohydrate diet and placed on the stock corn diet at weaning had the highest rate of flow. Also, the animals receiving the stock corn diet during pregnancy and thereafter have the lowest volume of saliva and the highest viscosity, and these animals had the highest incidence of caries. However, these relationships are not borne out in the other groups. It is evident that the salivary flow-dental caries relationship are complex, but it is interesting that in the groups in which the highest caries incidence is found occurs in the group with the lowest salivary volume and highest viscosity.

TABLE VIII

SALIVARY FLOW AND VISCOSITY IN RATS RECEIVING THE DIFFERENT DIETS

PREWEANING DIET	POSTWEANING DIET	MEAN RELATIVE VISCOSITY	MEAN FLOW (ML./MIN.)
CHO	CHO	2.16	1.75
	SCD	2.21	2.84
SCD	CHO	1.88	2.5
	SCD	2.31	1.35

SUMMARY

A significant decrease in dental caries incidence was obtained in animals born to mothers on a high sucrose diet when compared to similar animals born to mothers receiving a stock corn diet of parents receiving a stock corn diet prior to breeding.

In another study where the parents received a high sucrose diet for 150 days prior to breeding, no significant differences in dental caries were noted in the offspring. The two studies differed only in the preconception diet of the mothers. The influence of this factor on the caries susceptibility of the offspring needs further study. Its recognition and explanation may clarify the interpretation of some of the conflicting data in the literature.

Enamel solubility, salivary flow, blood glucose, blood chloride, blood calcium, and blood phosphorus, femur calcium, phosphorus or ash content tests were performed to determine if any of these variables would explain the dental caries data obtained in this study.

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RELATION OF STRUCTURE TO THE MICROHARDNESS OF HUMAN DENTIN

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THE HARDNESS of human dentin has been reported by a number of investigators using a variety of methods. Wright and Fenske[1] used an abrasion method which gave macroscopic results and reported that there was no relationship between hardness and anatomic position in dentin. They also reported that dentin in the teeth of females was significantly harder than in males and that the hardness of dentin in both approached a maximum at an age of 31 to 40 years. Hodge and McKay[2] used a microscratch method and stated that crown dentin was somewhat harder than root dentin, although the difference observed seems to be within the limits of experimental error. Zieger[3] reported that the dentin increased in hardness with age, and Richter[4] observed that dentin under areas of attrition or caries was harder than normal dentin. Likewise transparent dentin was reported by Proell and Schubert[5] to be harder than normal dentin.

Previous microhardness measurements from this laboratory[6] on crown dentin established an average Knoop hardness number (KHN) of 68. It was determined that the standard deviation for dentin was 5 KHN and that approximately 15 per cent of the hardness values had such large deviations from the average that they were considered to be outside the range of experimental error and to represent areas of different hardness. The largest variations in the hardness of dentin were observed in the transverse section just below the occlusal enamel surface and in the dentin near the dentinoenamel junction. Measurements in the latter area were generally discarded because unsymmetrical indentations were obtained as a result of uneven surfaces produced by polishing areas of different hardnesses (enamel—dentinoenamel junction—dentin). In this work it was not possible to relate hardness measurements to the histologic structure of dentin. There was some indication, however, of a relation between the histologic structure of dentin and the hardness.

The purpose of the present study is to correlate the microhardness of dentin with differences in structure. A modified Pollak trichrome staining technic[7] was selected to relate these hardness values to the structure of dentin. The staining method indicated areas worthy of investigation, thereby greatly reducing the number of hardness measurements required.

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EXPERIMENTAL

Specimen Preparation.—Recently extracted teeth were prepared for sectioning as previously reported.[6] The teeth embedded in plastic were sectioned, using a water-cooled alumina wheel, 0.33 mm. thick. The specimens were sectioned, as shown in Fig. 1, so that alternate sections 1 to 2 mm. and 0.1 mm. thick were obtained. The sections were numbered and hand polished by the successive use of 400A and 600A Norton Tufback speed wet paper supported on a plate-glass slab. In addition, the thick sections were polished with Shamva and CRO metallographic polishes at high speeds. The thin sections were subjected to the modified Pollak trichrome staining technic. In general, areas containing large amounts of organic material were stained dark purple to blue while the more highly calcified areas were stained pink to

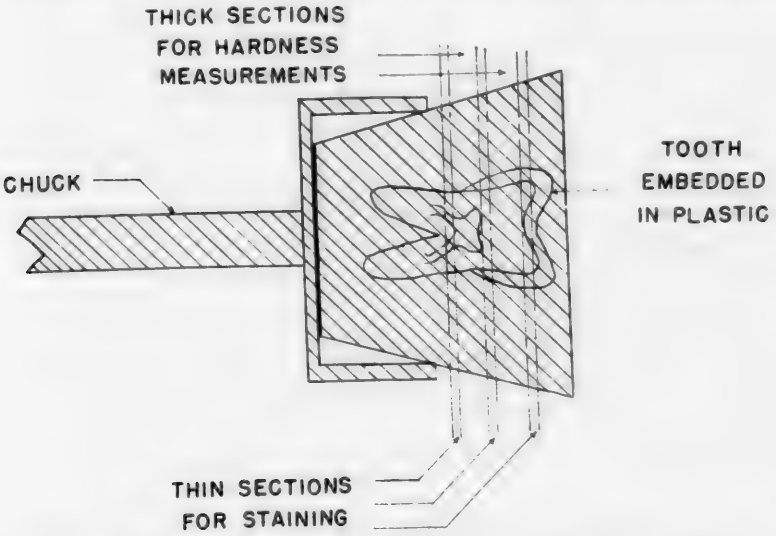

Fig. 1.—Sketch of an embedded tooth showing the positions for sectioning.

magenta (purple-red). Thus, hardness measurements could be made on the thick sections on the basis of the staining characteristics of the adjacent thin section. This procedure was necessary because the action of this stain alters the hardness of the dentin in the underlying as well as the surface layer.

Hardness Testing.—An "MO" Tukon microhardness tester with a Knoop diamond indenter was used with loads ranging from 2 to 25 grams. The measurements taken with the 10-gram load were found to show less experimental variation than those obtained with smaller loads and were considered to be more representative of microhardness values than those obtained with larger loads because of the heterogeneous nature of dentin. A normal load application cycle was used in all cases with the indenter being in contact with the specimen for 15 seconds. The ratio of the long to the short diagonal of the indentation was used as the criterion for accepting or rejecting a measurement.

Hardness measurements were made on transverse sections of 29 mature posterior and anterior teeth. Indentations were made in areas of a polished tooth section which was of interest on the basis of the amount of stain taken up by the adjacent thin section. It was difficult to observe histologic variations on the polished dentin, and hardness measurements were meaningless if taken on polished, stained specimens.

RESULTS AND DISCUSSION

Stained transverse sections in the crown area were generally magenta in color, being darker near the center of the section and having a lightly stained ring of dentin adjacent to the dentinoenamel junction. The dimensions of this ring varied from one tooth to another but generally the ring was approximately 0.5 mm. wide. The stained transverse sections just below the occlusal surface had a mottled appearance in the central area due to the variations in the distance from the specimen surface to the dentinoenamel junction. Sections in the center of the crown or root were sometimes mottled, the light areas indicating transparent dentin. A narrow ring around the pulp chamber in the root sections was stained magenta and denoted the predentin zone; the adjacent dentin was stained a dark red-purple, becoming more nearly magenta in color toward the cementum. The dark area appeared to represent an area where the dentin tubules were changing direction most rapidly. Carious areas in the dentin were also darkly stained.

Comparison of hardness measurements from one area of a tooth to another should be made within the same tooth since the hardness of dentin may vary considerably in similar areas of different teeth. Typical values for the average hardness of dentin in different transverse sections, 1 to 2 mm. apart, are listed in Table I. Thus, for a single tooth, the average microhardness values of various sections are the same.

TABLE I

MICROHARDNESS OF DENTIN IN VARIOUS SECTIONS OF A TOOTH

SECTION	NO. OF READINGS	AVG. KHN	MEAN DEVIATION
1	55	65	±7
2	59	68	±9
3	86	75	±9
4	90	66	±9

In general, hardness measurements made in the magenta-stained areas of the crown sections represented the average hardness and varied from 65 to 83 Knoop hardness numbers (KHN), while the lightly stained peripheral areas, near the dentinoenamel junction, were definitely softer, with values of 52 to 56 KHN. It should be mentioned that large differences in dentin tubule concentration were observed in crown sections but no measurable differences in microhardness were observed. Typical results obtained in crown sections are shown in Table II. Tooth A had a high average hardness of 83 KHN compared to teeth B, C, and D, which had average values from 69 to 72 KHN. These latter

values appear to more nearly represent the usual average hardness of dentin. The areas near the dentinoenamel junction in teeth A, B, C, and D had essentially the same hardness, the average values ranging from 52 to 56 KHN. The average deviations reported show that the average hardness values in these two areas do not overlap, but represent true differences in the hardness of the dentin. These results may be interpreted in the light of histologic information as resulting from the presence of interglobular dentin in the region of the dentinoenamel junction, the structure of which would be expected to be softer than the adjacent dentin because of the greater content of organic material. The lesser calcification of dentin in this area is also indicated by x-ray absorption[8, 9] through the presence of a radiolucent zone of lower calcification. In addition, the dentin tubules may have branches in this area which might imply that the dentin near the dentinoenamel junction would be softer than the adjacent dentin.

TABLE II
MICROHARDNESS VARIATION OF CROWN DENTIN

	CENTRAL CROWN DENTIN			DENTIN NEAR D-E JUNCTION		
TOOTH	NO. OF READINGS	KHN	MEAN DEVIATION	NO. OF READINGS	KHN	MEAN DEVIATION
A	16	83	±12	14	55	±7
B	4	72	±4	4	56	±3
C	15	72	±6	8	56	±4
D	8	69	±7	21	52	±7

Richter[4] reported that the crown dentin near the dentinoenamel junction was harder than the surrounding area, whereas Hodge[10] published data which showed that peripheral dentin near the dentinoenamel junction had the same hardness as the adjacent dentin. These conclusions do not agree with the observations in this study that the dentin near the dentinoenamel junction is softer than the adjacent areas. Their results may be explained by the problems involved in polishing such areas and the type of equipment used to obtain the previous hardness measurements.

The microhardness measurements obtained on a typical transverse root section are represented in Fig. 2. Knoop hardness numbers of about 34 were obtained near the pulp chamber. The hardness of the dentin increased as the distance from the pulp chamber increased and a consistent value (65 KHN) was obtained about 0.5 mm. from the pulp chamber. Inasmuch as the distance from the pulp chamber to the cementum was about 2 mm., most of the root dentin has the same microhardness as the dentin in the center of the crown. It it not surprising that the dentin adjacent to the root canal is softer than that of the surrounding areas because of the presence of less calcified predentin. X-ray and dye absorption experiments[11] have shown that the root canal dentin is more porous than the adjacent dentin, so that lower hardness values in this area would be expected.

Hodge and McKay[2] reported that dentin in the root of a tooth was softer than under the crown, while Wright and Fenske[1] found no relation between the hardness and position. The former authors' statement is true if one compares the crown dentin with the root dentin near the pulp chamber. However,

there is no difference between the hardness of dentin in the crown or root if the root dentin farther than 0.5 mm. from the root canal is compared with crown dentin farther than 0.5 mm. from the dentinoenamel junction. The latter authors possibly did not observe differences in hardness in different areas of dentin because of the lack of sensitivity of the abrasion method in evaluating microhardness.

Several of the transverse crown sections contained carious areas in the dentin and a few of the transverse root sections had areas of transparent dentin. Shown in Fig. 3 is a sketch of a typical transverse crown section containing a carious area. The Knoop hardness numbers indicate that the microhardness of the carious region is very low, 19 to 34 KHN, and that the hardness of the peripheral area around the carious region is between 81 and 85 KHN, which is

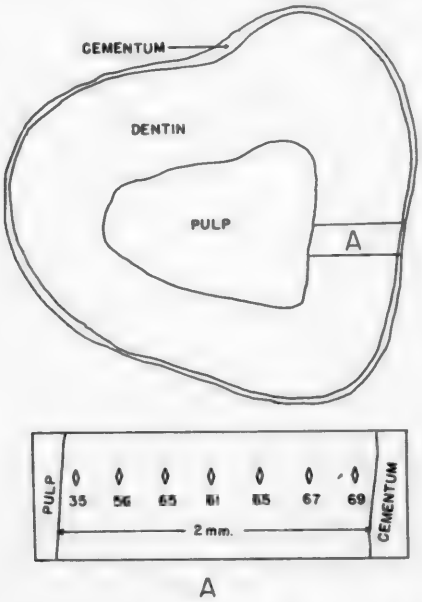

Fig. 2.—Microhardness variations from the pulp to the cementum in a transverse root section.

about 10 KHN harder than the dentin outside this peripheral caries area. Also, the hardness of the dentin near the dentinoenamel junction is lower than the surrounding areas. These measurements agree with those of Richter[4] and Hodge and McKay[2] which indicated that the dentin under areas of caries was harder than the surrounding dentin. This probably is caused by a repair mechanism which causes higher calcification of the dentin in areas surrounding caries.

The hardness of various areas in a transverse root section containing transparent dentin is shown in Fig. 4. The transparent dentin had a Knoop hardness of about 80 compared to a value of approximately 65 in adjacent areas. This result agrees with the data of Proell and Schubert[5] which showed

that transparent dentin was harder than normal dentin, and is not surprising because transparent dentin is more completely calcified. Again, the microhardness of the dentin near the pulp chamber was low, having values of 37 to 43 KHN.

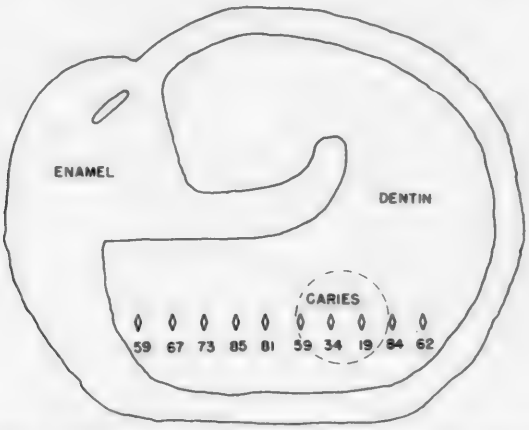

Fig. 3.—Microhardness measurements on a transverse crown section containing a carious area.

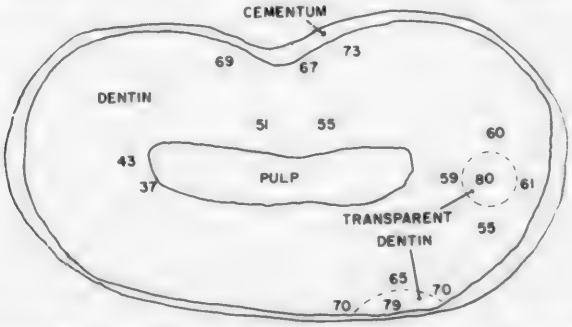

Fig. 4.—Microhardness in various areas of a transverse root section containing transparent dentin.

SUMMARY AND CONCLUSIONS

A modified Pollak trichrome staining technic on polished transverse tooth sections was found useful in indicating areas in dentin that might have different microhardness values.

Microhardness measurements in transverse crown and root sections of mature, freshly extracted, human teeth showed that the hardness of dentin near the dentinoenamel junction was about 15 KHN softer than the surrounding dentin.

The microhardness of dentin adjacent to the pulp chamber was about 30 KHN lower than the surrounding dentin.

Dentin in the center of the crown sections had the same hardness as the dentin away from the pulp chamber in the root sections.

Dentin surrounding caries lesions had a microhardness approximately 10 KHN greater than normal dentin, whereas microhardness values at the center of the lesion were much softer.

Transparent dentin was harder than the adjacent dentin by about 10 KHN.

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RESEARCH ANNOTATIONS*

AGE VARIATIONS IN THE NUMBER OF MAST CELLS IN ORAL MUCOSA AND SKIN OF ALBINO RATS

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THE determination of quantitative variations of mast cells in different tissues and under different conditions (for example, age variations) may help clarify the histophysiologic role of these cells. Previous investigations have shown a high number of mast cells in skin and mucous membranes (Wislocki and Sognnaes, Am. J. Anat. 87:239, 1950; Carranza and Cabrini, Oral Surg., Oral Med., & Oral Path. 8:1093, 1955).

Mast cells were counted in oral mucosa, nasal mucosa, and skin of stock albino rats of the following ages: 3 days (3 animals), one month (5 animals), 3 months (5 animals), and one year (4 animals). The material was fixed in formalin, which does not alter the number of mast cells in the rat (Asboe-Hansen, bull. d'histol. appliq. à la physiol. 27: 5, 1950), and processed simultaneously. Sections, 7 microns thick, were made and a total of 641 fields were studied. The results are given in Table I. Fields covering the surface given by objective ×40 and ocular ×12 were selected at random. Countings were made without reference to the age of the animal under study.

TABLE I

	3 DAYS	1 MONTH	3 MONTHS	1 YEAR
Skin	(a) 7.50	8.68	6.26	5.55
	(b) 2.83 ± 0.12	3.03 ± 0.11	2.60 ± 0.10	2.46 ± 0.11
	(30)*	(45)	(50)	(40)
Oral mucosa	(a) 0.96	5.26	3.91	2.78
	(b) 1.21 ± 0.08	2.40 ± 0.08	2.10 ± 0.07	1.81 ± 0.07
	(60)	(96)	(90)	(80)
Nasal mucosa	(a) 0.19	0.42	0.82	1.14
	(b) 0.83 ± 0.08	0.96 ± 0.08	1.15 ± 0.08	1.28 ± 0.10
	(30)	(40)	(40)	(40)

(a)—Mean.

(b)—Mean standard error of the mean. Each item was transformed according to the following formula $x' = \sqrt{x + 0.5}$ for the application of significance test "t" to Poisson distribution.

*Number of fields for each group are given in parentheses.

In skin, an increase in the number of mast cells of rats from 3 days to one month of age was noted ($P < 0.3$). After one month of age, the number diminished progressively (3 months, $P < 0.01$; one year, $P < 0.001$). The number of cast cells found at one year of age was lower than that found at 3 days ($P < 0.05$).

Oral mucosa (tongue and palate) showed a much more significant increase in mast cells from 3 days to one month of age ($P < 0.001$). The reduction after one month was also clear (3 months, $P < 0.01$; one year, $P < 0.001$). The number of mast cells found at one year of age was higher than that found at 3 days ($P < 0.001$). Nasal mucosa showed a progressive increase of mast cells from 3 days to one month ($P < 0.3$), 3 months ($P < 0.01$), and one year ($P < 0.001$).

The analysis of the findings revealed an increase in the number of mast cells from 3 days to one month in skin and oral and nasal mucosae, which coincides with connective tissue maturation, and suggests a relation between this and mast cells. After reaching the higher values at one month of age, the number of mast cells diminished gradually in skin, as shown by previous workers (Hellstrom and Holmgreen, Svenska Lakartidninger 44: 617, 1947). This reduction is more evident, however, in oral mucosa. Nasal mucosa, on the other hand, seems to increase continuously its content of mast cells, but absolute numbers of mast cells found in nasal mucosa are very low and their counting difficult.

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INCIDENCE AND DISTRIBUTION OF ORAL PLEUROPNEUMONIA-LIKE AND L ORGANISMS

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MORTON, Smith, Williams, and Eickenberg (*J. D. Res.* 30: 415, 1951), utilizing a medium containing bacteriostatic dyes, found that 51 of 110 human salivas contained pleuropneumonia-like organisms (PPLO). Since the medium utilized was not particularly suitable for the detection of L forms of bacteria, a study was made to determine the distribution of both oral PPLO and bacteria capable of reverting to L forms. For this survey, a liquid medium and culture technic was devised for the isolation and subculture of oral PPLO and L organisms (Gilmore, E., and Burnett, G. W.: *J. Bact.* 77: 147, 1959). By this technic, material from the oral cavity was cultured in a composite veal-thioglycollate (V-T) broth for no longer than 96 hours, after which the L organisms were cultured two or three times on penicillin-containing media in order to stabilize them. Numerous PPLO and L organisms were obtained by this procedure; the L organisms could then be propagated on suitable agar and in liquid media in stable form.

Three sources of material from human beings were cultured: (1) unstimulated saliva, (2) scrapings from teeth obtained with a sterile probe, and (3) carious material obtained from freshly extracted teeth. The various samples were inoculated into V-T broth containing 1 per cent serum fraction and 500 units of penicillin per milliliter. After 48 hours, material from the mid-portion of the anaerobic zone of the V-T broth culture was transferred to a PPLO agar plate (Difco) containing no penicillin and to a similar plate, with a center well, containing 0.15 ml. of a solution of 10,000 units of penicillin per milliliter. Cultures were incubated in a candle jar at 37° C. for 48 hours. After staining by Dienes' vital staining technic (*J. Infect. Dis.* 65: 24, 1939), PPLO and L type colonies were identified according to the criteria of Wittler, Cary, and Lindberg (*J. Gen. Microbiol.* 14: 763, 1956). Additional criteria for identification were the ability of an organism to grow as PPLO initially without penicillin and for L organisms to grow initially only in the presence of penicillin. However, due to the volume of cultures required, it was not possible to differentiate each PPLO from each L organism by determining reversion or nonreversion to bacterial form.

The following results were obtained.

SOURCE	NUMBER OF SPECIMENS	SPECIMENS CONTAINING PPLO AND L ORGANISMS	PERCENTAGE POSITIVE
Saliva	52	43	82.7
Tooth scrapings	33	31	93.9
Carious material	43	6	13.9

In each individual tested, either the saliva or tooth scrapings were positive so that 100 per cent of the oral cavities of the individuals tested contained PPLO or bacteria capable of transforming to L organisms. L organisms were more prevalent than PPLO. Fewer positive cultures were obtained on the penicillin-free plates than were found on the penicillin-containing plates, also indicating a predominance of L organisms in the oral specimens.

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THE CARIES-INHIBITING EFFECTS OF Na_2HPO_4 AND $CaHPO_4$

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RECENTLY, several investigators have demonstrated a reduction in the caries score of rats whose diets were supplemented with Na_2HPO_4 and, in some instances, with $CaHPO_4$. It was of interest to test the effectiveness of these salts under our laboratory conditions employing the NMRI-D caries-susceptible rat, a high protein, purified type of diet, and the grinding technic for caries scoring, as described by Schlack and his coworkers (NMRI Report, Project X-418, No. 5, July 17, 1947). For comparison, concomitant studies were performed, using a whole wheat flour diet which, contrary to the purified diet, was inadequate for optimal growth of the rats. The basal purified diet had the following percentage composition: casein, 30.0; sucrose, 63.8; HMW salt mixture, 2.0; complete vitamin mixture, 2.2; linoleic acid, 1.0; and corn oil, 1.0. The purified diet was supplemented with 1.5 per cent of either Na_2HPO_4 or $CaHPO_4$. The composition of the basal wheat flour diet "291" and of diets "292" (Na_2HPO_4 supplemented) and "293" ($CaHPO_4$ supplemented) are reported by McClure* and Muller (*J. A. D. A.* 58: 36, 1959).

Weanling male rats, between 25 and 40 grams in weight and 14 to 15 per group, were maintained as previously described (Losee, F. L., et al.: *J. D. Res.* 36: 904, 1957). After 5 weeks of ad libitum feeding, the rats were sacrificed. The results indicate that supplementation of the whole wheat flour diet with Na_2HPO_4 reduced the severity of carious attack but $CaHPO_4$ had no effect. However, in the case of the purified diet, the addition of either Na_2HPO_4 or $CaHPO_4$ resulted in lower caries scores.

DIET	CARIES SCORE	TYPE OF LESION (NO. PER RAT)		
		I	II	III
Purified	39.4 ± 1.3	10.7	12.7	1.1
Purified + Na_2HPO_4	29.4 ± 1.6	13.3	7.9	0.1
Purified + $CaHPO_4$	25.5 ± 0.9	11.7	6.9	0.0
291	29.2 ± 2.4	10.6	8.4	0.6
292 (Na_2HPO_4)	15.8 ± 1.8	12.4	1.7	0.0
293 ($CaHPO_4$)	29.4 ± 2.7	10.8	7.8	1.0

McClure and Muller have suggested that the presence of NaCl in the diet may be a determining factor in whether $CaHPO_4$ is a cariostatic agent. The wheat flour diet employed contained no added NaCl, whereas the HMW salt mixture used in the purified diet contained about 7 per cent NaCl. The present observations, along with the report of Barnard and Johansen, *J. D. Res.* 37: 34, 1958 (Abst.), that $CaHPO_4$ was cariostatic in two diets containing NaCl but not in a diet lacking NaCl, support the thesis of McClure and Muller and suggest a more thorough investigation of the role of this salt.

The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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PROCEEDINGS OF THE NORTHWEST BRANCH OF THE I.A.D.R.

OCTOBER 26, 1958

UNIVERSITY OF WASHINGTON, DENTAL SCHOOL, AND UNIVERSITY OF OREGON, DENTAL SCHOOL

1. A CLINICAL-LABORATORY EVALUATION OF THREE INTRACANAL ANTIBACTERIAL AGENTS.—*Bernard J. Zeldow, University of Washington, Dental School.* The selection of an intracanal medicament for root canal therapy has been the subject of numerous articles. Advocates of either nonspecific drugs as camphorated para monochlorophenol or polyantibiotics, such as PBSN, claim their choice as the better. The present investigation was designed to clarify the present status of intracanal medicaments.

A clinical laboratory investigation was undertaken in an unbiased effort to evaluate three root canal medicaments: camphorated para monochlorophenol (CPMP); polyantibiotics, penicillin-bacitracin-streptomycin-nystatin (PBSN); and polymyxin B-bacitracin-neomycin-nystatin (PBN[2]). The latter combination was selected because of its wide bacterial spectrum and infrequent parenteral use.

The drugs were evaluated on the basis of symptoms during treatment, and the number of visits to obtain a negative bacteriologic culture. Because of the potential inhibition of growth of organisms due to carry-over of the medicament, 5 ml. and 25 ml. tubes of inoculated media were incubated for 48 hours and one week. The results indicate the following:

1. There is no statistically significant difference in the effectiveness of the three intracanal medicaments tested.

2. Five milliliters and 25 ml. cultures, taken of the same individual, will agree about 85 per cent of the time at the end of both 48 hours and one week.

3. The agreement between the 48 hour and one week cultures in both the 5 ml. and 25 ml. tubes agreed about 90 per cent of the time for all three medicaments at the end of 48 hours and about 93 per cent of the time after one week.

2. THE NEED FOR UNIFORMITY OF ENDODONTIC INSTRUMENTS, EQUIPMENT, AND FILING MATERIALS.—*John I. Ingle, University of Washington, Dental School.* It has been established that an atrocious lack of uniformity of endodontic instruments and filling material exists today. This lack of formulation manifests itself in many objectionable ways in respect to endodontic cavity preparation and obturation. As a result, a dental manufacturing company has undertaken the challenge to produce root canal instruments and filling materials after criteria for standardization had been established and a new numbering system introduced.

Actual production samples of reamers, files, and silver points were measured on an electric microcomparator, accurate to 1 μ. An analysis of the

results indicated the following contributions: (1) establishment of a simplified number system based upon instrument formula, (2) with two exceptions, a consistent diameter and taper for all instruments and silver points, and (3) a consistent formula for size progression.

When a few technical problems are solved, a standardized total endodontic preparation may be finally realized.

3. Effect of Calcium Hydroxide on Carious Dentin.—*T. M. Lewis, University of Washington, Dental School.*

4. Certain Automatic Reactions of Children Under a Dental Stress.—*E. R. Schumacher, University of Washington, Dental School.*

5. Caries Incidence in a Group of Preschool and School Age Children.—*D. B. Law, University of Washington, Dental School.*

6. Demonstration of an Apparatus to Support a Theory for Hygroscopic Expansion of Dental Gypsum Materials.—*David B. Mahler, University of Oregon, Dental School.* A theory for the mechanism of hygroscopic expansion of dental gypsum materials is presented whereby water in contact with the setting gypsum material serves to reduce the expansion-inhibiting action of the disappearing liquid phase; this action being a manifestation of surface tension forces. An apparatus used to support this theory is described and its function demonstrated. It consists of automatically measuring and recording the expansion while controlling the addition of water to the setting gypsum material. The expansion is seen to stop upon shutting the water supply off and to start immediately upon opening the water supply. The absence of any time lag in this reaction is evidence of a force mechanism responsible for the phenomenon of hygroscopic expansion.

7. Direct Staining of Carious Teeth for Protein and pH Reactions.—*N. H. Rickles and R. M. Weigel, University of Oregon, Dental School.* The purpose of the experiment was to determine, if possible, the protein and pH reactions of carious teeth, particularly at the periphery of the carious lesions. To this end, 518 teeth were subjected to ninhydrin staining for protein, and bromeresol green for pH reactions. Varying concentrations for and methods of exposure of the stains to the teeth were employed. These teeth were freshly extracted, quick frozen or treated in vivo. The dye was introduced into the pulp chamber and/or into the excavated carious areas of some teeth and these areas were subsequently sealed off with wax. Other teeth were split down the middle or sectioned into thin slices, after which they were placed into the stain or combinations of stains.

The control teeth showed no acid reaction anywhere while the dentin stained evenly for protein. No matter what technic was used, carious teeth showed acid reactions at the periphery of the lesions when bromeresol green was used and/or an intensely staining protein reaction with ninhydrin. When both stains were used simultaneously, evidence of increased acidity and proteolysis in intimate relationship to each other was seen, although the intense protein stain often overshadowed the more delicate indicator change. However, in a number of cases, an acid zone was seen preceding the protein. Several possible explanations for the increased protein reaction are suggested.

8. An Apparatus for Stimulating Human Dentin by Temperature Change.—*D. J. Anderson and J. D. Godfrey, University of Oregon, Dental School.* This apparatus was designed to provide a sudden temperature change at the surface of dentin exposed in a prepared cavity. It consists of two

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concentrically placed needles with their ends ground flat, the inner needle being 3 mm. shorter than the outer needle. The inner needle is connected at its upper end to a constant temperature water source and it carries thermocouple leads which have their junction at its tip. The outer needle is connected at its upper end to a vacuum pump by a tube in which there is a side-arm closed by a solenoid valve. While this valve is closed, water flowing down the inner needle is sucked back along the outer needle, but when the vacuum is broken by opening the valve, water flows from the tip of the outer needle onto the surface to be stimulated. It is possible with this apparatus to record the threshold temperature at which a sensation is evoked from dentin and, in addition, the latent period between stimulation and response. Preliminary results are reported.